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# HUMAN BIOCHEMISTRY

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*WITH NINETY-THREE TEXT ILLUSTRATIONS  
AND FIVE COLOUR PLATES*

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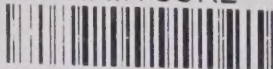
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TO  
~~MY WIFE~~ MOTHER





## Preface to Fourth Edition

Biochemistry continues to advance at great speed and in many directions. In this revision, as before, the author has attempted to keep sight of its vast new horizons. The first objective has of course been to eliminate any concepts which have changed or are of doubtful nature. More difficult has been the task of selecting from the almost limitless researches those advances which seem to be authenticated and at the same time of significant value to the student. From the many pages and volumes of current biochemical literature, it has been necessary to cull those discoveries which are either of proved worth or indicative of exciting trends. The original purpose of *Human Biochemistry*—to create a useful, understandable, and compact volume—has been kept in mind constantly.

Every chapter has been examined critically. In most cases this has been done by an expert in the field, whose advice has been followed as closely as possible. Large areas have been completely rewritten, notably those dealing with blood coagulation, enzymes and coenzymes, physiological oxidations, cholesterol metabolism, urea formation, transmethylation, and the mechanism of insulin action. There has been extensive revision of the sections concerning the tricarboxylic acid cycle, coenzyme A, formation of gastric HCl, biochemistry of tumors, the role of vitamin A in vision, vitamin B<sub>12</sub> and folic acid, electrolyte and acid-base balance, and the metabolism of a number of the amino acids. Pentose and fatty acid metabolism have been given more space, and the use of isotopes is reflected in many chapters.

Among the new topics thought worthy of discussion are the dextrans, triiodothyronine, glucagon, serotonin, the carbonic anhydrase inhibitors, blood iodine, lipoic acid, and the structure of insulin and of oxytocin. A section is devoted to the nomenclature of the steroids.

There have been introduced two new photomicrographs and a number of new figures and diagrams. Some of the latter have appeared elsewhere and are reproduced by kind permission of the authors and publishers. Several illustrations which appeared in the third edition have been modified to agree with current concepts.

The writer expresses his thanks for searching criticism of various chapters or large sections to Dr. Maurice M. Black, Dr. Richard J. Block, Dr. Halvor N. Christensen, Dr. Adam A. Christman, Dr. Charles L. Fox, Jr., Dr. C. E. French, Dr. David Glick, Dr. Franklin Hollander, Dr. Alfonso A. Lombardi, Dr. Walter Menaker, Dr. Walter H. Seegers, Dr. Sam Seifter, Dr. J. A. Stekol, and Dr. R. W. Swift. It is a pleasure to acknowledge the valuable counsel given by Dr. Stefan Ansbacher, Dr. Harry Barowsky, Dr. William H. Beinfeld, Dr. Lyman C. Craig, Dr. Harry J. Deuel, Jr., Dr. Louis B. Dotti, Dr. David L. Drabkin, Dr. Leonard G. Ginger, Dr. Sam Granick, Dr. Charles Haig, Dr.

William B. Langan, Dr. L. Corsan Reid, Dr. Kurt G. Stern, Dr. Carleton R. Treadwell, Dr. Vincent du Vigneaud, Dr. D. Wright Wilson, and Miss Rachel Reed. Continuous aid has come from the writer's associates, Dr. Harry Baron, Dr. Paul Fodor, Mr. Arthur Katchman, and Mr. Sherman Beychok. The advice and encouragement of his colleague, Professor Carl Neuberg, have been particularly gratifying. Thanks are expressed to all of the above and to many others who have sent in criticisms and suggestions. All of their ideas could not be embodied in this work, but for those which have been included (as well as for omissions) the writer assumes full responsibility, despite the fact that he could not hope to be an authority on so many phases of the subject.

Grateful mention should be made of the efficient secretarial assistance of Mrs. Ruth Glantz and the bibliographic work of Mrs. Eugenia Dover. The original line drawings are the work of Miss Natalie Pearlstein, and the photomicrograph of hemin crystals is by Mr. Jacob Glenner, to both of whom thanks are due.

ISRAEL S. KLEINER

New York, N. Y.



## Preface to First Edition

It is not so many years since physiological chemistry was essentially a pure science course in medical schools and reference to clinical applications was incidental if not accidental. Medical and dental students, reasonably enough, questioned the necessity of the subject in the curriculum, feeling it was not much more than a mental exercise, as Latin is so often considered in the academic curriculum. But those days have passed. The name biochemistry has now, in most instances, replaced the term physiological chemistry, and that bit of streamlining has been accompanied by a modern approach on the part of the chemistry faculty. The biochemist has come halfway from the laboratory toward the clinic.

The student now is shown the subject as an integral part of the practice of medicine—not just as a part of the medical curriculum. He learns that advances in every branch of medicine, surgery, and dentistry have been made as a result of biochemical research, that the human body is applied biochemistry, that the entire field of physiology is a series of biochemical reactions and pathological phenomena result from disturbances of these same reactions, and that biochemical discoveries are more and more responsible for progress in diagnosis and therapeutics. The present volume is an attempt to bring home to the student these clinical aspects of biochemistry without usurping any clinician's domain and without neglecting the fundamentals.

Since the preparatory years of medical and dental students are today being curtailed in many instances, it is necessary to keep in mind the fact that the classes for a few years will include some who are not so well equipped as one would wish. It is hoped that this text is neither too advanced for them nor too elementary for students who are well prepared.

The question of mentioning investigators' names and quoting references has given the writer considerable concern. However, consideration has been given primarily to the student and secondarily to the instructor or advanced student. Therefore, the number of such references has been kept as low as possible. The student should be familiar with the names of some of the scientists who are responsible for fundamental discoveries. There should also be available to him references to the more recent experimental studies which seem to be establishing new trends. The instructor may want to ask the student to go to the original sources, particularly if the subject is controversial. Many such references have been given, and reviews or monographs have been cited to permit a more extended study of some of the topics discussed, but it has been impossible to mention more than a small fraction of the names of competent investigators and authorities. In many instances, distinguished names have been omitted in order to improve the "readability" of the text.

Thanks are due to the following biochemists, physiologists, and specialists in other fields who read and criticized parts of the manuscript and who made

many valuable contributions: Dr. Alfred Angrist, Dr. Cameron V. Bailey, Mr. Harry Baron, Dr. Maurice M. Black, Dr. Richard J. Block, Dr. W. R. Bloor, Dr. Robert K. Brewer, Dr. Otis M. Cope, Dr. Louis B. Dotti, Dr. Charles Haig, Dr. Franklin Hollander, Dr. Arthur Knudson, Dr. William B. Langan, Dr. Joseph I. Linde, Dr. Fritz Lipmann, Dr. C. N. H. Long, Dr. Edgar G. Miller, Jr., Dr. Victor C. Myers, Dr. Marie O'Donahoe, Dr. Arnold H. Schein, Dr. Arthur H. Smith, Dr. Eric G. Snyder, Dr. Francis D. Speer, Dr. Henry Tauber, Dr. Abraham White.

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No textbook can be original. It is in the nature of a compilation of fundamental facts and recent advances. As a result it is built upon the work of many others which have preceded it. The present work is no exception. Many textbooks, reviews, and original articles have been consulted and their contents digested and assimilated into this volume. Acknowledgment is hereby made of this debt to many authors. Some copyrighted material has been used with the generous consent of the various authors and publishers.

New York

ISRAEL S. KLEINER

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# HUMAN BIOCHEMISTRY



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## Chapter 1

### INTRODUCTION

Biochemistry is the study of the chemical composition of living matter and the changes which occur in it. Such changes under normal conditions are termed physiological; abnormally they are pathological. Both phases are important. It is self-evident that a knowledge of the normal should precede a consideration of the abnormal. However, the pathological phases must not be neglected. Indeed, they are important not only for so-called practical reasons, but also because the study of deviations from the normal very frequently sheds light on the normal. Several occasions to study important examples of this principle will arise.

### PROTOPLASM

Living matter or protoplasm is impossible to define adequately. It differs from lifeless material in possessing the capabilities of growth, repair, and reproduction. These properties may not be apparent at all times in the same degree, but they are present to some extent in all living organisms. Moreover, the life processes go on at comparatively low temperatures and with great rapidity. Comparable reactions in the laboratory require high temperatures, often with increased pressure, or else they go on very slowly and quite incompletely. Many reactions of the living cell are of great complexity—intricate interwoven oxidations, disintegrations, and syntheses—in comparison with which the manifold simultaneous operations of a modern newspaper press are like simple mechanical toys. Some of these marvelous reactions are known and partly understood. Many others are only appreciated because of our awareness of the end products. We must be impressed by the orderly way in which all of the chemical activities of the body coordinate. This may be another attribute of living matter, the orderliness of its chemical reactions.

Protoplasm is composed of water, inorganic salts, and organic compounds. Water is undoubtedly the most important compound in tissues. The water of the tissues and body fluids is mostly in the free state, that is, substances may be dissolved in it and it may pass back and forth from blood to tissues, in and out of cells. A small fraction of the water is believed to be bound, that is, some of the water in hydrophilic colloid systems is combined so that the activity of the water molecules is reduced considerably. Free water varies according to diet and physiological activity, whereas bound water is a rather constant constituent of the tissues.

### Water of Tissues

In general, the more active the tissue from a physiological standpoint, the higher the content of water. Table I illustrates this point. True osseous tissue, the function of which is support and protection rather than physiological activity, has a low water content. The same is true of dentine, a hard protective but inactive tissue. Adipose tissue also is low in water; it too is inactive, being a food store and, in some instances, a protective or supporting material. An apparent exception is cartilage, a rather inert tissue with a high content of water. However, water is necessary in this instance to give the tissue its softness and pliability.

TABLE I  
CONTENT OF WATER IN TISSUES

TISSUE	PER CENT	TISSUE	PER CENT
Muscle	75-80	Pancreas	73
Nervous tissue	76	Adrenal	80
Heart	79	Thyroid	80
Kidneys	81	Thymus	77
Liver	75	Skin	70
Spleen	77	Adipose	10-30
Bones (with marrow)	46	Bones (without marrow)	23
Dentine	10		

The study of water balance will be taken up in a later chapter. Suffice it to say that we have several mechanisms for maintaining and controlling the water supply of the tissues. When these go wrong, a number of pathological states may ensue. Dehydration is a condition not at all uncommon and is likely to have a fatal outcome if not recognized and combatted. Edema is another—a condition in which fluid leaves the blood stream and accumulates in the tissues. Sometimes what appears to be a very minor disturbance results in a major catastrophe.

Water is needed for many and varied reasons. It is the solvent, the agency which enables water-soluble, water-miscible, or emulsifiable substances to be transferred in the body, not only in the blood, which is more than four-fifths water, but also inter- and intracellularly. Ionization takes place in water and ionization is a prerequisite to many biochemical reactions.

In the regulation of body heat, water is most important because of its peculiar physical properties. It possesses high specific heat. This enables the body to store heat effectively without greatly raising the temperature. It has high heat conductivity. This permits heat to be transferred readily from the interior of the body to the surface. Finally, it possesses high latent heat of evaporation, which causes a great deal of heat to be used in the evaporation of water, and thus cools the surface of the body. These are physical properties made use of by the body in the physiological regulation of body temperature.

### Inorganic and Organic Constituents

About 1 per cent of the total weight of an average tissue is ash, or inorganic salts. They comprise chiefly salts of the following cations:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ ,

$\text{NH}_4^+$ ; and of the anions:  $\text{Cl}^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ . Some of these may be linked to organic radicals, as is also the case for Fe, I, Cu, Zn, Mn, and some others. Except for water and small amounts of gases, such as  $\text{O}_2$  and  $\text{CO}_2$ , the balance consists of organic compounds: carbohydrates, lipids, proteins, and many others. The various tissues differ in all of these constituents qualitatively and quantitatively. It is to be expected that a nerve cell will not have the same composition as a salivary gland cell. However, all cells resemble each other chemically to some extent.

Since every cell contains a nucleus, it must contain nuclear constituents, and it also has a small number of primary constituents which resemble those of every other cell in a general way. However, they differ both qualitatively and quantitatively. They also differ in the content of certain special constituents which are peculiar to individual organs, and indeed to specialized parts of these organs. For example, a cell of adipose or fatty tissue will have a large proportion of inert fat which is characteristic of that tissue but also a small amount of protoplasm and a nucleus. The protoplasm and nucleus will resemble chemically the protoplasm and nucleus of a cell of, say, the gastric mucosa, but they must differ from each other in some ways because one has the function of laying down fat, while the other produces some constituent of the gastric juice.

### Classification of Biological Elements

Sixty of the ninety-two elements believed to be present in the universe occur in biological matter. Only a comparatively few, however, are found invariably, and some of these only in minute amounts. Fearon classifies the biological elements as follows:

#### A. Invariable or Constant Constituents

##### 1. Invariable Primary Elements

Hydrogen, carbon, nitrogen, oxygen, and phosphorus. These are found in all forms of life and make up the greater part of all tissues.

##### 2. Invariable Secondary Elements

Calcium, magnesium, sodium, potassium, iron, sulfur, and chlorine. These elements are also essential to life but are present in smaller amounts.

##### 3. Invariable Microconstituents

Minute amounts of copper, boron, silicon, manganese, fluorine, and iodine are said to be present in all forms of life.

#### B. Variable or Inconstant Constituents

##### 1. Variable Secondary Elements

These are elements which are found in fairly high concentrations in certain species, but they probably do not occur in all. Zinc, titanium, vanadium, and bromine are in this group.

##### 2. Variable Microconstituents

Lithium, rubidium, cesium, silver, beryllium, strontium, cadmium, germanium, tin, lead, arsenic, chromium, cobalt, nickel, aluminum, molybdenum, and barium have been found in minute amounts in one or more species.

##### 3. Contaminants

In still smaller amounts and of a more accidental occurrence are the contaminants argon, helium, mercury, thallium, selenium, bismuth, and gold.



## Isotopes

A number of the elements occur in nature as mixtures of the more common type with varying amounts of other forms having a slightly different atomic weight. These differing forms of the same element are called *isotopes*. Thus hydrogen with an atomic mass of 1 has an isotope *deuterium* with an atomic mass of 2; ordinary chlorine with an atomic mass of 35.457 has been found to be a mixture of two isotopes, the first and more abundant one having an atomic mass of 35 and a second whose atomic mass is 37. They have the same chemical properties, although they differ in atomic mass.

Exceedingly interesting and important biological investigations have employed the isotopes of several elements in recent years. The more uncommon isotope may be introduced into a definite group of a given molecule of a food-stuff, for example, in place of the common one. Then the fate of that group can be followed throughout its travels in the animal because of the special properties of the isotopic atom, particularly radioactivity. In this way, the carbon of a carboxyl or of a methyl group can be "tagged" and followed, or the hydrogen or nitrogen of an amino group. Radioactive isotopes of carbon, calcium, phosphorus, sulfur, iodine, iron, cobalt, and zinc are now available. However, it is frequently preferable to use a stable isotope in animal experimentation rather than a radioactive one, since radioactivity may be harmful. For example, radioactive carbon,  ${}^6\text{C}^{14}$ ,\* has a half-life† of 5,100 years and consequently would radiate in the animal's body as long as it was retained.  ${}^6\text{C}^{13}$ , however, is not radioactive and is, therefore, harmless. By such methods it has been shown that our biochemical reactions are much more dynamic and continuing than had previously been supposed. Many types of molecules must first be incorporated into protoplasm before they can be used or even excreted, and this is a continual process characteristic of living matter. The details of biochemical interactions are slowly but surely being worked out.

## References

- Fearon, W. R.: A Classification of the Biological Elements, *Scient. Proc., Roy. Dublin Soc.* 20: 531, 1933.  
Kamen, M. D.: Tracers, *Scientific American* 180: (No. 2): 30, 1949.  
Sacks, J.: *Isotopic Tracers in Biochemistry and Physiology*, New York, 1953, McGraw-Hill Book Co.

\*The subscript number indicates the atomic number of the element and the superscript the atomic mass. In biochemical literature the subscript is frequently omitted.

†Half-life, or half-life period, is the time necessary for the original concentration to be reduced one-half.



## Chapter 2

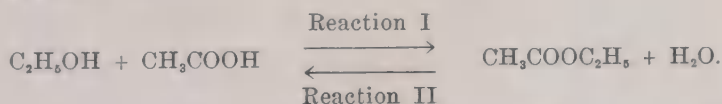
### PHYSICAL CHEMISTRY

Many biochemical problems can be explained only on a physicochemical basis, and biochemists are becoming constantly more aware of this fact. A brief summary of a few physicochemical topics and other fundamentals, therefore, is presented here.

#### LAW OF MASS ACTION

The law of mass action applies to the state of equilibrium existing in reversible reactions taking place in dilute solutions. This law states that the rate at which a reaction takes place, at constant temperature, is proportional to the product of the concentrations of the reacting substances. The concentrations are expressed as gram moles per liter, and such concentrations are represented by bracketing the symbols of the substances in question.

The reaction between ethyl alcohol and acetic acid to form ethyl acetate and water is a reversible reaction:



According to the law of mass action, the velocity of the reaction proceeding toward the right (Reaction I) depends upon the product of the concentrations of alcohol and acetic acid. That is:

$$V_1 \propto [\text{C}_2\text{H}_5\text{OH}] \times [\text{CH}_3\text{COOH}]$$

where  $V_1$  represents the velocity of Reaction I. Therefore,

$$V_1 = k_1 \times [\text{C}_2\text{H}_5\text{OH}] \times [\text{CH}_3\text{COOH}]$$

where  $k_1$  is a constant.

In a similar manner Reaction II proceeds at a velocity  $V_2$  which is proportional to the concentrations of ethyl acetate and water; that is,

$$V_2 \propto [\text{CH}_3\text{COOC}_2\text{H}_5] \times [\text{H}_2\text{O}]$$

and also

$$V_2 = k_2 \times [\text{CH}_3\text{COOC}_2\text{H}_5] \times [\text{H}_2\text{O}]$$

where  $k_2$  is another constant. Now, at equilibrium, Reaction I must necessarily proceed at the same rate as Reaction II, otherwise it would not be in equilibrium, and

$$V_1 = V_2 \text{ or}$$

$$k_1 \times [\text{C}_2\text{H}_5\text{OH}] \times [\text{CH}_3\text{COOH}] = k_2 \times [\text{CH}_3\text{COOC}_2\text{H}_5] \times [\text{H}_2\text{O}]$$

and, by algebraic division, we arrive at

$$\frac{[\text{CH}_3\text{COOC}_2\text{H}_5] \times [\text{H}_2\text{O}]}{[\text{C}_2\text{H}_5\text{OH}] \times [\text{CH}_3\text{COOH}]} = \frac{k_1}{k_2} = K_{\text{equil.}}$$

The new constant,  $K$ , is the equilibrium constant of the reaction. This constant will always be the same for a given reaction after equilibrium has been established, no matter what the proportion of the reactants may have been at the start. There will, of course, be a different constant for every reaction and the constant varies with temperature and pressure. Here we are interested mainly in the electrolytic dissociation constants.

If  $K$  is always the same for this reaction at equilibrium, then if the equilibrium is upset by adding or removing any of the four reacting substances, the system will tend to balance these substances until a new equilibrium is reached and  $K$  is reconstituted. For example, if more ethyl alcohol is added, more of it will combine with acetic acid to form more ethyl acetate and water.

To make the equation more general, we may say that for the reversible reaction



Using the same symbols as on the preceding page,

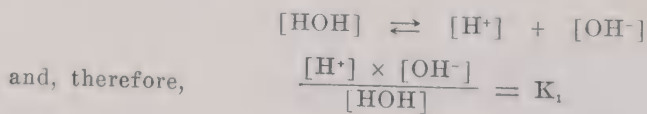
$$\begin{aligned} V_1 &= k_1[A]^a \times [B]^b \\ \text{and } V_2 &= k_2[C]^c \times [D]^d. \end{aligned}$$

At equilibrium,

$$\begin{aligned} k_1[A]^a \times [B]^b &= k_2[C]^c \times [D]^d \\ \frac{[C]^c}{[A]^a} \frac{[D]^d}{[B]^b} &= \frac{k_2}{k_1} = K_{\text{equil.}} \end{aligned}$$

## HYDROGEN ION AND HYDROXYL ION CONCENTRATION

Pure water is only very slightly dissociated. There is, however, a certain definite concentration of hydrogen ions, which, though small, must be balanced by the same concentration of hydroxyl ions. This state of affairs is reflected in the low but measurable conductivity of water. The dissociation of water may be represented by the following equation:



In this equation the denominator, undissociated water, is extremely large when compared with the numerator and may be considered a constant. The analogy may be drawn between this and a ship which has sprung a leak when in mid-ocean. The amount of water pouring into the ship's hull is of great moment, even though it is an infinitesimal part of the ocean, which to all intents and purposes remains constant. Here the numerator is the volume of water passing into the ship and the denominator is the constant ocean; therefore,

$$\begin{aligned} \frac{[\text{H}^+] \times [\text{OH}^-]}{K_2} &= K_1 \\ [\text{H}^+] \times [\text{OH}^-] &= K_1 K_2 = K_w \end{aligned}$$

$K_w$ , the dissociation constant for water, is, as would be expected, an extremely small value. It has been determined to be 0.000,000,000,000,01, or  $1/100,000,000,000,000$  or  $1/10^{14}$  at  $25^\circ \text{C}$ .; that is,

$$[\text{H}^+] \times [\text{OH}^-] = K_w = 1/10^{14} = 1 \times 10^{-14}$$

But, in pure water, the concentration of hydrogen ions must equal that of hydroxyl ions; therefore, since  $[\text{H}^+] = [\text{OH}^-]$ , it can be substituted for  $[\text{OH}^-]$ .

$$[\text{H}^+] \times [\text{H}^+], \text{ or } [\text{H}^+]^2 = 1 \times 10^{-14},$$

and taking the square root of both sides of the equation,

$$[\text{H}^+] = 1 \times 10^{-7}.$$

That is, the hydrogen ion concentration of water is  $1 \times 10^{-7}$  grams per liter, or  $1/10,000,000$  grams per liter. Either of these methods of expression is unwieldy and consequently Sørensen suggested that the negative exponent with its sign changed to positive be used and be termed "pH." Another way of stating this is

$$\text{pH} = -\log_{10}[\text{H}^+].$$

The pH of water, or neutrality, then is 7.0. If acid is added to water, the concentration of hydrogen ions, of course, will increase, and instead of  $1/10,000,000$  grams of  $\text{H}^+$  per liter, there would be a greater value with a *smaller* denominator. The pH of acidic solutions, accordingly, is less than 7.0, and that of alkaline solutions is greater than 7.0. Using  $\text{HCl}$  and  $\text{NaOH}$  solutions as examples, and assuming complete ionization, the relationship of acidity and alkalinity to pH and to pOH is shown in Table II. (In practice, pOH is seldom referred to.)

TABLE II  
RELATIONSHIP OF ACIDITY AND ALKALINITY TO pH AND POH

NORMALITY*	pH		pOH
0.1 N HCl	1	Acidity	13
0.01 N HCl	2		12
0.001 N HCl	3		11
0.0001 N HCl	4		10
0.00001 N HCl	5		9
0.000001 N HCl	6		8
0.0000001 N (= water)	7	Neutrality	7
0.000001 N NaOH	8	Alkalinity	6
0.00001 N NaOH	9		5
0.0001 N NaOH	10		4
0.001 N NaOH	11		3
0.01 N NaOH	12		2
0.1 N NaOH	13		1

\*A normal solution is one which contains 1 gram equivalent of the substance per liter. For further discussion, see under Titratable Acidity.

Examples of the conversion of hydrogen ion concentration to pH and vice versa are the following:

A. Given the hydrogen ion concentration,  $[\text{H}^+]$ , 0.00634M; to find the pH.

It is first convenient to express the concentration of hydrogen (or hydronium) ions as a whole number multiplied by 10 raised to the power indicated. Thus

$$[\text{H}^+] = 0.00634\text{M} = 6.34 \times 10^{-3}.$$

Since  $\text{pH} = -\log_{10}[\text{H}^+]$ , and  $[\text{H}^+]$  is the molar concentration, the logarithm must first be obtained.

$$\begin{aligned}\log [\text{H}^+] &= \log(6.34 \times 10^{-3}) = \log 6.34 + \log 10^{-3} \\ &= 0.8021 + (-3) = -2.1979\end{aligned}$$

To get the  $-\log$ , multiply both sides of the equation by  $-1.0$ .

$$-\log [\text{H}^+] = \text{pH} = 2.20 \text{ (pH is never expressed beyond the second decimal)}$$

B. Given the pH, 2.20; to determine the  $[\text{H}^+]$ .

$$\begin{aligned}\text{By definition } \text{pH} &= \frac{1}{\log[\text{H}^+]} = -\log [\text{H}^+] \\ (1) \text{ and } [\text{H}^+] &= 10^{-\text{pH}} \\ \text{From (1)} \quad [\text{H}^+] &= 10^{-2.20} \\ \text{or } [\text{H}^+] &= 10^{-3.0} \times 10^{+0.8} \\ x &= 10^{0.8} \\ \log x &= 0.8 \\ x &= 6.31 \\ \text{Therefore, } [\text{H}^+] &= 6.31 \times 10^{-3.0} \text{ or } 0.00631\text{M}.\end{aligned}$$

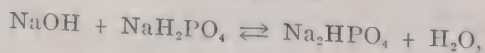
Hydrogen ion concentrations are determined either by electrometric methods or by the use of standard buffers and indicators. A description of the electrometric methods is beyond the scope of this volume. However, it should be pointed out that the instruments have been skillfully developed and simplified to such a degree that pH determinations of great accuracy may now be made in a few minutes. For an understanding of the indicator method, there must first be a brief discussion of buffers, which are of interest also from the physiological viewpoint.

### Buffers

A buffer solution is one which tends to maintain a constant hydrogen ion concentration when acid or alkali is added to it. A buffer system usually consists of a weakly dissociated acid and the salt of that acid, or a weak base and its salt. For example, carbonic acid and sodium bicarbonate constitute a buffer system. If acid is added to  $\text{NaHCO}_3$ , the following reaction occurs:



A *strong* acid, HCl, which might be expected to raise the hydrogen ion concentration, reacts with a weak base in such a way as to yield a weak acid,  $\text{H}_2\text{CO}_3$ , and a neutral salt. The hydrogen ion concentration has not been raised appreciably. Also, if NaOH is added to  $\text{NaH}_2\text{PO}_4$ ,

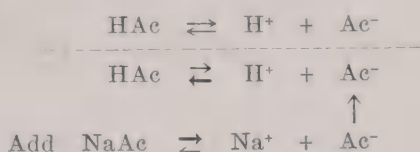


the weakly acid sodium dihydrogen phosphate buffers the *strong* alkali by yielding the weakly alkaline disodium hydrogen phosphate. Again the hydrogen ion concentration has not been changed very much. In the first instance, the buffer system or buffer pair  $\text{H}_2\text{CO}_3$  and  $\text{NaHCO}_3$  results and the  $\text{H}_2\text{CO}_3$  is effective in



buffering alkalis. In the second instance,  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  become the buffer pair, also effective in buffering in either direction. Both of these systems, and several others as well, operate in the body to prevent marked changes of hydrogen ion concentration from taking place, and they are remarkably efficient.

In such systems it is evident that the "common ion effect" is operative. That is, if one adds to a weak electrolyte a strong electrolyte having an ion in common with the weak electrolyte, the ionization of the weak electrolyte is diminished, and the concentration of the ion not in common is also lessened. For example, if sodium acetate is added to acetic acid, the ionization of the acid will be repressed, resulting in a decreased  $[\text{H}^+]$ .



The addition of the acetate ion (from the sodium acetate) which is in common with the acetate ion from the acetic acid tends to drive the equilibrium to the left. As a result, the concentration of hydrogen ion is decreased by recombination with the acetate ion to form undissociated acetic acid.

The hydrogen ion concentration of most body fluids and secretions is on the alkaline side. Urine may be acid and gastric juice is very acid, but these are exceptions. Many influences tend to change this alkalinity, but the buffers present prevent marked fluctuations in hydrogen ion concentration. The pH of blood, for example, stays within the limits 7.3 to 7.5 in health. When these limits are exceeded, we have a condition of acidosis or alkalosis with alarming symptoms and, frequently, dire results. In Table III are given some of the pH values for various human fluids.

TABLE III  
pH VALUES OF HUMAN BODY FLUIDS AND SECRETIONS

Blood	7.4	Pancreatic juice	8.0
Milk	6.6-6.9	Intestinal juice	7.7
Bile	7.8	Cerebrospinal fluid	7.4
Urine	6.0	Saliva	7.2
Gastric juice	0.87	Aqueous humor of eye	7.2
(parietal secretion)			

It is quite difficult to keep a solution at constant pH if no buffer is present because of the influence of the  $\text{CO}_2$  of the air or the alkali of the glass container or because of other influences. Consequently, buffers are frequently required. Various mixtures have been prepared consisting of definite amounts of the acid, or base and its respective salt. Some of these sets of buffer systems are shown in the appendix. Since such buffer sets maintain their pH indefinitely, they are used in the indicator method of determining pH.

### Indicators

An indicator is a weakly ionized acid or base, having one color in the un-ionized state and another when ionized. If the indicator is a weak acid and

is, let us say, red when undissociated and blue when dissociated, it will be red when distinctly acid and blue when distinctly alkaline, since the alkaline salts of a weak acid are highly dissociated in solutions. At some point in between—which is a different pH for each indicator—half the indicator will be present in acid form and half as the alkaline salt. At this pH this indicator will be purple. There will accordingly be a range of color changes on each side of this mid-point, and each gradation of color will correspond to a different pH. The more acid, the more red and less blue; the more alkaline, the more blue and less red.

If we represent this indicator as HInd, since it is a weakly dissociated acid, the reaction would be a reversible one:



According to the law of mass action, we then have

$$\frac{[\text{H}^+][\text{Ind}^-]}{[\text{HInd}]} = K.$$

K is the dissociation constant of this indicator. Since this is a weak acid, the  $[\text{H}^+]$  and  $[\text{Ind}^-]$  must necessarily be small. If, now, a strong acid is added, the  $[\text{H}^+]$  becomes much greater and the denominator,  $[\text{HInd}]$ , must increase to keep K constant. This is why it will be less dissociated on the acid side of this indicator. On the alkaline side, the reverse occurs because the  $[\text{H}^+]$  decreases and the undissociated  $[\text{HInd}]$  likewise must decrease to keep K constant; that is, the undissociated acid diminishes in concentration and the  $[\text{Ind}^-]$  increases, thus intensifying the alkaline color.

### Indicator Method of Determining Hydrogen Ion Concentration

Since buffer mixtures of definite and constant pH are available, they can be used as standards to which the indicators are added. The colors so produced will be definite for a given pH with a given indicator. Unknown solutions then may be treated with the indicator chosen and the color compared with the

TABLE IV  
INDICATORS

INDICATOR	pH RANGE	COLOR CHANGE
Acid cresol red	0.2- 1.8	Red-yellow
Thymol blue (acid range)	1.2- 2.8	Red-yellow
Dimethylaminoazobenzene	2.9- 4.0	Red-yellow
Bromphenol blue	3.0- 4.6	Yellow-blue
Congo red	3.0- 5.0	Blue-red
Methyl orange	3.1- 4.4	Orange red-yellow
Bromeresol green	4.0- 5.6	Yellow-blue
Methyl red	4.2- 6.3	Red-yellow
Litmus	4.5- 8.3	Red-blue
Alizarin red	5.0- 6.8	Yellow-red
Bromeresol purple	5.4- 7.0	Yellow-purple
Bromthymol blue	6.0- 7.6	Yellow-blue
Phenol red	6.6- 8.2	Yellow-red
Neutral red	6.8- 8.0	Red-yellow
Cresol red	7.2- 8.8	Yellow-red
Metacresol purple	7.6- 9.2	Yellow-purple
Thymol blue (alkaline range)	8.2- 9.8	Yellow-blue
Phenolphthalein	8.3-10.0	Colorless-red
Alizarin yellow	10.0-12.0	Colorless-yellow
Tropaeolin O	11.1-12.7	Yellow-orange
Acyl blue	12.0-13.6	Red-blue



colored buffer standards. The various indicators have a comparatively narrow range of color change, and preliminary tests with indicator papers or rough tests with several indicators or with a "universal indicator" may be necessary before the actual determination is made. Definite quantities of the unknown and of each of a series of buffers are treated with a certain amount of the indicator. The buffers usually comprise a series having differences of 0.1 pH from each other. Thus a graded series of colors is obtained and the unknown solution has the pH of the one it matches most closely. A set of useful indicators with their pH ranges and color changes is given in Table IV.

**The Henderson-Hasselbalch Equation.**—Approximate values of the pH of buffer solutions may be calculated if the composition of the mixture, as well as the ionization constant of the weak electrolyte is known; for example, in the case of acetic acid,

$$\begin{aligned}\text{HAc} &\rightleftharpoons \text{H}^+ + \text{Ac}^-, \\ \frac{[\text{H}^+] \times [\text{Ac}^-]}{[\text{HAc}]} &= K_{\text{Ac}}, \\ \text{or } [\text{H}^+] &= K_{\text{Ac}} \frac{[\text{HAc}]}{[\text{Ac}^-]}.\end{aligned}$$

In a mixture of the acid and its salt, say NaAc, most of the acid is un-ionized. Consequently the  $[\text{HAc}]$  is about the same as the total acid concentration and  $[\text{H}^+]$  would be extremely small. Also, since the salt is completely ionized, the value  $[\text{Ac}^-]$  is approximately equal to the total salt concentration. Therefore, for approximate calculation, the equation may be written as follows:

$$[\text{H}^+] = K_{\text{Ac}} \frac{\text{Acid}}{\text{Salt}}, \text{ or } K \frac{\text{Acid}}{\text{Salt}} \text{ to make the equation applicable to other salts and acids.}$$

Taking the negative logarithm of this,

$$\begin{aligned}-\log [\text{H}^+] &= -\log \left( K \times \frac{[\text{Acid}]}{[\text{Salt}]} \right) \\ &= -\log K + \left( -\log \frac{[\text{Acid}]}{[\text{Salt}]} \right).\end{aligned}$$

Since  $-\log [\text{H}^+]$  is called pH, we may call  $-\log K$  by the term pK; therefore,

$$\text{pH} = \text{pK} + \log \frac{[\text{Salt}]}{[\text{Acid}]}.$$

That is, the pH of a buffer solution is determined by the logarithm of the ratio of salt to acid and by the pK (i.e., the negative logarithm of the ionization constant of the acid). This last equation is known as the Henderson-Hasselbalch equation.

It finds many uses in chemical calculations. Examples follow:

(1) To calculate the pH of a buffer solution which contains 0.1 mole of sodium acetate and 0.1 mole of acetic acid at 25° C.  $K_{\text{a}} = 1.8 \times 10^{-5}$

$$\begin{aligned}\text{pH} &= \text{pK}_{\text{a}} + \log \frac{[\text{Salt}]}{[\text{Acid}]} \\ \text{pK}_{\text{a}} &= -\log K_{\text{a}} = -\log (1.8 \times 10^{-5}) \\ &= -(\log 1.8 + (-5)) = 4.74\end{aligned}$$

$$\text{pH} = 4.74 + \log \frac{[0.1]}{[0.1]}$$

$$\text{Since } \log 1 = 0, \quad \text{pH} = 4.74$$

(2) To calculate the pH of two solutions, one of which is unbuffered and the other buffered, before and after adding the same amount of a strong base to each.

- (a) Given a solution of HCl, the concentration of which is 0.0001 molar; to determine the pH.

$$[\text{H}^+] = 0.0001 = 1 \times 10^{-4}$$

$$\text{pH} = 4.0$$

Now add 0.0001 moles of NaOH. To determine the pH after this addition. Since the NaOH added exactly neutralizes the acid present,

$$\text{pH} = 7.0.$$

- (b) Given the buffered solution of (1), containing 0.1 mole of sodium acetate and 0.1 mole of acetic acid, with a pH of 4.74. To calculate the pH after adding the same amount of NaOH; namely, 0.0001 mole.

$$\text{pH} = 4.74 + \log \frac{0.1 + 0.0001}{0.1 - 0.0001}$$

(Because there has been added 0.0001 mole of salt [numerator] and the same amount of acid has been subtracted [denominator].)

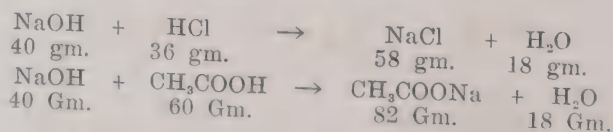
$$\begin{aligned} \text{pH} &= 4.74 + \log \frac{0.1001}{0.0999} = 4.74 + \log 0.1001 - \log 0.0999 \\ &= 4.74 + 0.0008, \text{ or } 4.74 \end{aligned}$$

These calculations (2a and 2b) show also the resistance of a buffer to a change in the pH. A small quantity of base, added to an unbuffered solution, produces a change of three pH units, whereas an equivalent amount of base added to a buffered solution causes an insignificant change.

### Titratable Acidity

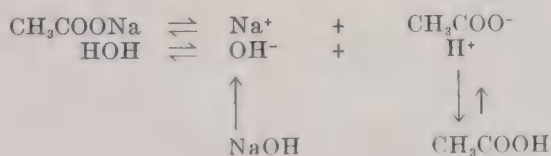
Indicators are used also in the determination of titratable acidity or alkalinity. Titratable acidity is total acidity, or total potential acidity. On the other hand, the hydrogen ion concentration might be termed the true acidity. Let us consider two different acids, hydrochloric and acetic, having the same normality. By normality is meant the concentration as related to that of a normal solution, and a normal solution is one containing 1 gram equivalent of a substance per liter of solution. For example, a normal solution of HCl is one which will contain 36 grams of HCl per liter; i.e., one which contains 1 gram of hydrogen per liter. A normal NaOH solution contains 40 grams of NaOH per liter; i.e., a solution which will combine with one gram of hydrogen ion per liter. Similarly a normal acetic acid solution has 60 grams of acetic acid per liter of solution. In the case of divalent, trivalent, etc., acids, bases, and salts, one must divide the molecular weight by 2, 3, etc., respectively.

Now, if we wish to combine a gram equivalent weight of NaOH with either HCl or  $\text{CH}_3\text{COOH}$ , we find, of course, that it requires a gram equivalent weight of either of these acids.

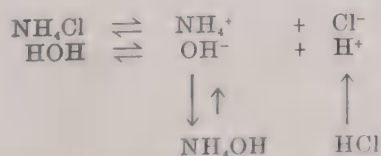


If, now, the 40 grams of NaOH in each case are dissolved and diluted to 1 liter and the 36 grams of HCl and 60 grams of  $\text{CH}_3\text{COOH}$  are each dissolved in sufficient water to make 1 liter, we have made up normal solutions of each. One liter of the Normal HCl will neutralize 1 liter of Normal NaOH, and 1 liter of Normal  $\text{CH}_3\text{COOH}$  will neutralize 1 liter of Normal NaOH. Thus Normal or N/1 HCl is equivalent to N/1  $\text{CH}_3\text{COOH}$  because each is potentially capable of yielding the same amount, namely, 1 gram of hydrogen ions. The acetic acid is not nearly as strong an acid as the hydrochloric acid; that is, it is not as greatly dissociated as HCl. However, if one adds NaOH to it (the N/1 NaOH, for example), little by little, the small number of hydrogen ions is neutralized by the base and more and more acid dissociates until finally all the hydrogen ions have been displaced. This process is known as titration. A Normal (N/1), tenth Normal (N/10), etc., solution of any acid then is equivalent to any other N/1, N/10, etc., acid, volume for volume. Again, an N/10 NaOH solution will neutralize an N/10 acid, volume for volume.

The *stoichiometric point* in an acid-base titration is the point at which an equivalent amount of base has been added to the acid. The end point of such a titration, however, will not necessarily be at a pH of 7.0. This will depend upon the salt formed by the reaction. Obviously we are interested in the pH of the solution at the point at which the maximum amount of salt is present. In titrating HCl with NaOH, it will be the exact point at which all of the chloride ion is balanced by the sodium ion and we have a solution of NaCl. Since this is a neutral salt with no buffering action, many different indicators may be used to tell the end point, since one additional droplet of alkali above the equivalent amount will cause a great change in the pH. However, in titrating a weak acid, like acetic acid, with a strong base, like NaOH, the salt formed at the stoichiometric point will be sodium acetate, which has an alkaline reaction. This is seen from the following reaction generally referred to as hydrolysis:



That is, as sodium acetate dissociates in the presence of water, the strong basic reaction overbalances the weak acidic one. Therefore, in this titration an indicator which changes at an alkaline pH; e.g., phenolphthalein, must be used. Similarly, in titrating a weak base such as ammonium hydroxide with a strong acid such as hydrochloric acid, we finally get ammonium chloride at the stoichiometric point. This has an acid reaction and requires an indicator which changes on the acid side, such as methyl red.





## THE COLLOIDAL STATE

In 1861, Graham classified all substances into two categories, depending on their ability to pass through parchment and similar membranes. Since those substances which diffused readily were those which easily crystallized, such as copper sulfate, sucrose, etc., he designated them "crystalloids." Those which did not pass through, e.g., gelatin, starch paste, glue, etc., were considered to be noncrystallizable and were called "colloids," from the Greek word meaning "glue." These terms continue to be used, although we now are able to crystallize many of the colloids, and the crystalloids can be converted to a colloidal form. The modern conception of these differences is based on the size of the particles dispersed in the water or other medium. Colloidal particles are large; they cannot pass through the pores of ordinary parchment or collodion membranes. However, they are not large enough to settle out by gravity, as suspensions are, or to float at the top of the medium, as imperfect emulsions do. In true solutions, so-called crystalloidal solutions, the mixture is homogeneous; the constituents are present in the molecular or ionic state and are uniformly distributed throughout and among the molecules of water or other solvent. Colloidal systems are heterogeneous, that is, there are two "phases"—the finely divided particles and the medium in which they find themselves. By "phase" is meant a physically distinct portion of matter. The particles are called the *dispersed phase* and the medium, usually a fluid, is the *dispersion medium*. Both phases may be solids, liquids, or gases, with a single exception, which is that it is not possible to have a colloidal dispersion of a gas in a gas. Smoke is a solid dispersed in a gas, fog is a liquid dispersed in a gas, and froths and foams are gases dispersed in liquids. We are more concerned with liquids dispersed in liquids, liquids dispersed in solids, and solids dispersed in liquids.

The size of the particles in colloidal systems is generally stated to be from 1 millimicron ( $1\text{ m}\mu$ ) to 100 millimicrons, but it must be understood that it is not possible to set arbitrary limits at either end. In fact, the tendency is to place the upper limit somewhat higher, at say  $500\text{ m}\mu$  ( $0.5\text{ }\mu$ ). One millimicron is one one-millionth of a millimeter ( $0.000,001\text{ mm.}$ ). Particles having smaller diameters than  $1\text{ m}\mu$  are molecular or ionic, and if much above  $100\text{--}500\text{ m}\mu$ , they are coarse enough to settle out. The smallest colloidal particles, therefore, are but little larger than crystalloidal molecules, and the largest ones are nearly the size of the particles in a suspension.

Colloidal particles may be removed from the dispersion medium by forcing the fluid, under pressure, through an appropriate membrane. This is termed *ultrafiltration*. By using membranes of varying porosity, it is possible to separate different colloids from each other and to estimate the size of colloid particles. A colloid particle is often termed a "micelle."

Ultracentrifugation is another method of removing colloid particles. By centrifuging at a very high speed, the dispersed phase may be separated from the dispersion medium. Substances in true solution cannot be separated from their solvents by these two methods. Still another procedure is electrophoresis. (See page 113.)

A simpler method than any of those described is dialysis. This will be discussed later in this chapter.

### Types of Colloids

Colloids may be grouped into two main classes, depending on their ability to take up the dispersion medium. The *lyophilic colloids* (emulsoids) have a great attraction for the dispersion medium; in fact, each particle has a layer of the dispersion medium surrounding it. The *lyophobic colloids* (suspensoids) contain *no* such layer. The names are quite descriptive, lyophilic meaning solvent-loving and lyophobic meaning solvent-hating. The lyophilic colloids include starch, egg albumin, blood proteins, soap, and gelatin. This is the more important type physiologically. Examples of the lyophobic colloids are the colloidal metals, such as gold, silver, platinum, etc. Both types exist in the fluid state as *sols*. Many lyophilic colloids form semi-solid *gels*. A well-known example is gelatin, which, when dilute, is fluid. When a moderately high concentration is allowed to stand, it sets into a jelly or gel. This gel may be converted into its sol by warming and, on cooling, it may again gel; that is, the change from a sol to a gel is frequently reversible. However, this is not always the case, as we shall see when we study coagulation and denaturation of proteins. When a gel forms, apparently long chains of molecules of the colloid interlace and entrap the fluid by capillary forces. The gel then is really a liquid dispersed in a solid.

Some sol-gel transformations take place at constant temperatures. For instance, if a colloidal iron oxide sol is allowed to stand quietly, it will set into a gel. Upon shaking, a sol is reformed. This phenomenon is known as "thixotropy." Protoplasm is said to have thixotropic properties.

**Preparation of Colloidal Solutions.**—Various organic compounds, such as gelatin, starch, and soaps, form sols or colloidal solutions, as they are frequently called, simply when added to water. Other colloidal solutions may be formed by chemical reaction. For example, the reduction of gold chloride yields the lyophobic colloid, colloidal gold. Mechanical grinding in a colloid mill may also be used to reduce a substance to such a fine state that it may readily be dispersed in colloidal form. The dispersal of any solid into the colloidal state is called "peptization." For example, the dispersal of gelatin in water is known as its peptization and water is the peptizing agent, but of course other liquids or solutes may also be peptizing agents.

### Electrical Charges on Colloids

Dispersed colloidal particles carry electrical charges, the dispersion medium carrying the opposite charge. Colloidal gold particles carry negative charges, the positive charge being in the water adjacent to them. Since bodies carrying like charges repel each other, this serves to keep the colloids dispersed and is one of the factors which make for the stability of a colloidal system. If an electric current is sent through a colloidal system, the colloid will pass to the anode if it is electronegative or to the cathode if it is electropositive. This is called *cataphoresis*. Furthermore, a colloid may



be precipitated by adding a colloid of opposite charge, thereby neutralizing the charge and upsetting the stability of the dispersed substance. A few examples of positively and negatively charged colloids of both the lyophilic and lyophobic classes are as follows:

LYOPHILIC	LYOPHOBIC
Proteins in neutral or alkaline solution (-)	Gold (-)
Proteins in acid solution (+)	Platinum (-)
Starch (-)	Stannic oxide (dispersed by HCl) (+)
Soaps (-)	Sols of metallic oxides and hydroxides and basic dyestuffs (usually +)
Aluminum hydroxide (+)	
Gum acacia (-)	

Under certain conditions the phenomenon of *coacervation* may be observed. When two lyophilic colloids of opposite electric charge are mixed, they may not precipitate or flocculate in the ordinary sense, but because of the hydration shell on each, they may form microscopic droplets. These droplets, after a while, may coalesce to form a viscous, fluid layer. This is a coacervate. It contains the two colloids which are held apart by the hydration shells, and each colloid retains its own electric charge. Therefore, if the pH is changed or an electrolyte is added to a coacervate, it may again form a sol of the original lyophilic colloids. Many phases of protoplasm are believed to be coacervate in nature. Thus, vacuole formation closely resembles a phenomenon seen when complex coacervates are permitted to age.

**Tyndall Effect.**—A colloidal solution, such as a dilute starch solution, appears slightly cloudy or opalescent to the eye. If a beam of light is passed through it, the beam becomes visible as a much cloudier path, particularly if viewed against a dark background. This is the Tyndall phenomenon and is the same phenomenon that one observes when a beam of sunlight enters a darkened room. In this instance the minute particles of dust in the air deflect the light. In the case of the colloidal solution, it is the colloidal particles which partly diffract the light and diffuse it. By means of an ultramicroscope or a dark-field microscope, the Tyndall effect becomes visible in another way. In these instruments light is sent through a drop of solution in a horizontal direction. Visible particles reflect light to the observer's eye and can be seen as shining objects. Invisible particles, such as colloids, may be seen as dancing bright specks. They dance and dart in *Brownian movement*, just as visible particles do. The reason is that they are under constant bombardment from molecules of the dispersion medium. A large particle is likely to be hit by about the same number of bombarding particles from each side at the same instant. The smaller the particle, the less the chance of instantaneous and equal striking from all sides. It will move whenever it receives an unequal number of blows from different directions.

The nephelometer is an instrument which measures the Tyndall effect. Substances which form extremely fine precipitates in suspension may be estimated quantitatively by means of it.

**Stability of Colloids.**—This constant movement of particles is one of the forces which tends to keep a colloid stable. It keeps the colloidal particles distributed throughout the system, rather than allowing them to settle or rise. The

size of the colloidal particle is another factor, since the smaller its size, the more closely it approximates molecular dimensions and therefore more nearly resembles a true solution. This is not always the case, however, since the lyophobic colloids may have extremely small dimensions and they are, in general, less stable than the lyophilic colloids. The reason for this is that the suspensoids, the lyophobic, have little or none of the dispersion medium attached, or adsorbed, to their surfaces, and therefore the particles may approach each other closely enough to permit the mass attraction to overcome the repulsion due to the electric charge. The lyophilic colloids have a layer of the fluid adsorbed which makes them more stable, because this keeps them farther apart. The electric charge present on the colloid, as a stabilizing factor, has already been discussed.

Any procedure which tends to diminish the effect of one or more of the stabilizing factors will tend to precipitate the colloid. Thus, as said before, the addition of another colloid of opposite electrical charge will do so. In the case of the lyophobic colloids, a small amount of electrolyte accomplishes this by also neutralizing the charge. This does not occur in the case of the lyophilic colloids, but large amounts of electrolytes will precipitate them. Probably this effect, "salting out," occurs as the result of dehydrating the surface layer of fluid. Furthermore, violent agitation, freezing and thawing, heating, all can "break" colloidal solutions by modifying one or more of the stabilizing factors.

Although lyophobic colloids may be precipitated by the addition of small amounts of electrolytes, this may be hindered by the presence of small quantities of a lyophilic colloid. This "protective" action is believed to be accomplished by the adsorption of the lyophilic colloid onto the surface of the lyophobic colloid, thereby preventing the electrolyte from easily reaching it. It has been thought that the protective action of colloids present in bile and urine might be a major reason for the prevention of the precipitation of almost insoluble constituents of these fluids. Conversely, when the protective colloidal action is not effective, gallstones and kidney stones result. Lange's colloidal gold test is based on this protective colloidal action. Colloidal gold is a bright orange red sol. The addition of normal cerebrospinal fluid does not precipitate it. Abnormal cerebrospinal fluid may do so, the result showing in various shades, depending upon the degree of precipitation. The test is performed under exact quantitative conditions, and curves are obtained which are indicative of pathological states (see Chapter 8).

### Surface Reactions of Colloids

The dispersed phase of a colloid differs from a suspension of solid matter in that the colloid is subdivided into very much smaller particles. It is apparent that each suspended particle has a surface. Similarly, each colloidal particle has surface, and although each particle is far smaller than a unit of a suspension, the number will be much greater for the same weight and therefore the total area of surface will be greater. This can be illustrated by a simple example. A cube of any material 1 cm. on a side has a surface area of  $6 \times 1 \text{ cm.}^2$ , or  $6 \text{ cm.}^2$ . If



it is divided into eight cubes, each edge measuring  $\frac{1}{2}$  cm., the total area will be  $\frac{1}{2}$  cm.  $\times$   $\frac{1}{2}$  cm.  $\times$  6 sides  $\times$  8 cubes = 12 cm.<sup>2</sup> The subdivision of each small cube is continued, and the amount of surface is doubled each time each cube is cut into eight smaller ones. Eventually, when the cubes are down to a size comparable with colloidal dimensions, i.e., 100 m $\mu$  on a side, the total of all the tiny cubes will be 600,000 cm.<sup>2</sup>, that is, 100,000 times the original surface area, produced by simply subdividing the cube. This indicates the enormous area presented by the surfaces of colloid particles. Upon these surfaces substances present in solution may be adsorbed and become concentrated. Adsorption is the phenomenon in which there is condensed upon a surface a layer of ions, molecules, or aggregates of molecules that are present in the medium with which it is in contact. The amount of adsorption depends upon the extent of surface exposed and the specific nature of the surface and of the substance adsorbed upon it. Furthermore, the degree of adsorption is increased by a rise in pressure and is diminished by a rise in temperature. Many physiological phenomena are surface reactions. The enzymes, which catalyze so many reactions of the body, probably act through surface forces. They are colloids and the substances they act upon are probably adsorbed by them as a first step in the chemical action which is brought about.

### Surface Tension

The surface of a liquid behaves as if it were a stretched elastic film. This is due to the unbalanced attraction of the molecules to each other. According to Laplace, the molecules of a solution are strongly attracted to each other, but only over a very short distance. It is probably greatest at a distance equal to about the diameter of a molecule. Fig. 1 illustrates and explains this phenomenon. Molecules *C*, *D*, *E*, and *F* are not at the surface and are attracted equally in all directions. They are therefore able to move freely in all directions. Molecules *A* and *B*, however, are at the surface (*XY*) and are not attracted upward because of the absence of molecules of the fluid above the surface. Consequently they tend to be drawn downward and pulled sideward, and the layer of surface molecules is thereby stretched. The effect of this "film" is seen in the tendency of drops of water and mercury and soap bubbles to assume a spherical form because of this cohesive pull sideward and inward. This phenomenon occurs at any surface or "interface" which separates a liquid from air or other gases or which separates one liquid from another. This explanation is a simplification of this phenomenon; there are other forces involved than that of attraction.

Surface tension may be measured in a number of ways, perhaps most conveniently with a stalagmometer. This is a pipette of special design with a capillary tube ending, permitting a measured amount of fluid to flow out drop by drop. The number of drops will depend on the size of the drops, which, in turn, varies with the surface tension. A comparison with the number of drops of pure water permits one to calculate the surface tension of the solution. Surface tension is expressed as ergs per square centimeter or dynes per centimeter. For very accurate work great precautions of cleanliness must be taken, since

small amounts of some substances alter the surface tension materially. Soaps, oils, proteins, and salts of the bile acids reduce the surface tension of water, while sodium chloride tends to increase it. These and similar effects aid in explaining some physiological actions; for example, fat digestion and absorption. Substances which reduce surface tension accumulate in the surface film and are said to be adsorbed, whereas the reverse is true of those which increase it. There is a stalagmometric method for the determination of bile acids in bile, which has been used as a liver function test, based on the fact that an important function of the liver is the secretion of bile acids.

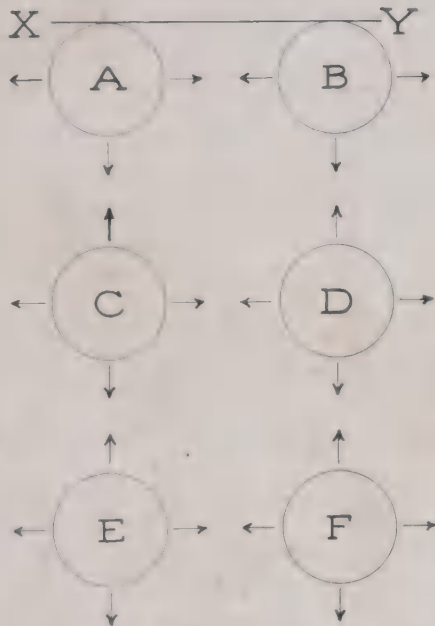


Fig. 1.—Diagram to explain surface tension. Molecules A and B are at the surface and are not attracted upward because of the absence of molecules above them. Consequently they tend to be drawn downward and sideward, and the layer of molecules at the surface is thus stretched. Molecules C, D, E, and F are not at the surface and are attracted equally in all directions.

### Gas Laws

Since substances in solution behave like gases in some respects, it is necessary at this point to remind the student of the gas laws.

1. *Boyle's Law.* At a constant temperature the pressure is inversely proportional to the volume.

2. *Gay-Lussac's or Charles' Law.* With constant pressure, the volume will increase  $1/273$  for every degree rise in temperature above  $0^{\circ}\text{C}$ .

3. *Avogadro's Law.* At the same temperature and pressure, molecular weights of all gases occupy the same volume.

4. *Henry's Law.* The amount of gas which a liquid will dissolve is directly proportional to the pressure of the gas in contact with it. This holds only for gases which do not react chemically with the solvent. It is especially important in connection with changes in atmospheric pressure.

The amount of nitrogen which blood plasma and other body fluids will dissolve will increase as atmospheric pressure increases. Consider, for example,



the case of a deep-sea diver. During descent he is subjected to air under high pressure. The nitrogen does not enter into any chemical reactions and, in accordance with Henry's law, goes into solution in the aqueous media of the body. As the diver rises to the surface, the pressure will decrease and nitrogen will come out of solution in bubble form. These nitrogen bubbles may form in capillaries or in nervous tissue and result in decidedly unphysiological effects. The same may take place as an aviator rises from the comparatively high pressure on the earth's surface to the low pressures up above the surface. This is one of the major problems of military medicine.

5. *Dalton's Law.* The pressure exerted by a mixture of gases is equal to the sum of the separate pressures which each gas would exert if it alone occupied the whole volume. This law is referred to in explaining respiratory phenomena.

6. *Graham's Law.* The rate, or speed, of diffusion of a gas is inversely proportional to the square root of its density. Since the density of a gas is directly proportional to its molecular weight, it follows that the law may also read: The rate of diffusion of a gas is inversely proportional to the square root of its molecular weight.

## DIFFUSION, OSMOSIS, AND DIALYSIS

**Diffusion.**—If a strong solution of a salt, such as copper sulfate, is placed in a glass vessel and a layer of distilled water is carefully poured over it, the blue copper sulfate will be seen to pass up gradually into the colorless water until finally the entire body of fluid has the same color. This process is called *diffusion*. The velocity with which this occurs depends on the size of the particles of the substance in solution. Thus, Prussian blue, being composed of very large particles, will diffuse more slowly than the copper sulfate. Higher temperatures also speed up the process. It should be observed that diffusion involves the passage of substances, in true solution or in colloidal solution, through the fluid in which they find themselves. In the many fluids of the body, within cells, during secretory activity, diffusion must be constantly occurring.

**Osmosis.**—Osmosis is the passage of a solvent through a semipermeable membrane. Such a membrane is permeable only to the solvent, not to the solute; i.e., the substance in solution. The classical experiment of Pfeffer illustrates the point. He precipitated copper ferrocyanide in the walls of an unglazed porcelain jar by filling the jar with potassium ferrocyanide solution after immersing it in a solution of copper sulfate. Such a film of copper ferrocyanide permits water to pass through but does not allow certain soluble substances; e.g., sugars, to do so. Consequently, when such a jar, fitted with a glass tube into which the liquid can rise, and filled with sugar solution, is placed in distilled water, water will pass through the semipermeable membrane into the sugar solution until the column of diluted sugar solution is no longer increased. The pressure which would have to be exerted to prevent the rise of the column of sugar solution is called the osmotic pressure.

The osmotic pressure of a solution is directly proportional to the concentration of the solute. Strong salt solutions will have higher osmotic pressures than

weak ones. Therefore, 1 gram of salt dissolved in 100 ml. will have twice the osmotic pressure of 1 gram of salt dissolved in 200 ml. In other words, the osmotic pressure is *inversely* proportional to the volume, showing that Boyle's law is applicable to solutions. Similarly, it has been found that the osmotic pressure will increase  $1/273$  for each rise of  $1^\circ$  C. (Gay-Lussac's law). Avogadro's law also applies to osmotic pressure since all solutions containing the same number of dissolved particles will have the same osmotic pressure at a constant temperature. That is to say, the osmotic pressure depends on the number of particles dispersed in the fluid. This is independent of the nature of the particles. Consequently, a given number of ions, undissociated molecules, and aggregates of molecules (colloidal particles) in identical volumes of fluid will all have the same effect. Since each ion has the same effect as a molecule, it is evident that an electrolyte such as sodium chloride, which furnishes two ions, will have twice as high an osmotic pressure when completely dissociated as a non-ionized substance of the same molecular weight. It is also seen that since large aggregates of molecules have the same effect as ions or small molecules, colloidal "solutions" will have low osmotic pressures because of the comparatively small number of such particles present.

Although the osmotic pressure can be determined by the use of such an apparatus as is illustrated in Fig. 2, in practice it is measured by indirect means. The boiling point of a solvent is raised by the addition of a solute, and the freezing point is lowered similarly. The amount of either change is proportional to the concentration of the particles of the solute and, as we have seen, the same holds true for osmotic pressure.

Solutions which have the same osmotic pressure are said to be *isosmotic*. If a cell is in contact with a solution having the same osmotic pressure as the cell contents, the amount of water passing into the cell is balanced by that passing out, provided that the cell membrane is impermeable to the solutes. In this case the solution is not only isosmotic but also *isotonic*; that is, the cell volume is unchanged; its "tone" is maintained. If the osmotic pressure of a solution of the same solutes is greater than that of the cell, it is hyperosmotic and is said to be *hypertonic*, and water will pass from the cell to the solution. If it is lower, it is hypo-osmotic and is *hypotonic*, and water will flow into the cell. In a hypertonic solution the cell will shrink, and in a hypotonic solution it will swell. We have presupposed in each case that the solutes do not permeate the membrane. If, however, one or more of the solutes can pass through the membrane and is not present in the same concentration within the cell, it is evident that an isosmotic solution may not be isotonic. Osmotic pressure is not the same as tonicity. However, physiologically they are about synonymous, and it is found that they usually parallel each other rather closely.

Thus, 0.9 per cent NaCl solutions are isotonic to human red blood corpuscles, and if the two are in contact the amount of water passing into the cell is balanced by that passing out. A solution of lower salt concentration will have lower osmotic pressure than the cell. It is hypotonic. If red blood cells are immersed in hypotonic solutions, water passes from the more dilute to the more concentrated solution (i.e., from the lower to the higher osmotic pres-

sure) in so great an amount that the red cells will swell and may even burst. Hypertonic solutions will cause water to pass out of the red cells and cause them to become shriveled or *crenated*. Therefore, solutions used for injection are made up to be isotonic; otherwise unphysiological effects would be produced on the corpuseles. Similarly, whenever cut tissues or viscera are bathed with fluid, isotonic solutions are used since they cause less change in the cells with which they come in contact. The body adjusts its various fluids to approximately the same osmotic pressure. For example, the osmotic pressures of such extremely divergent types of fluids as blood, hepatic bile, pancreatic juice, and lymph collected simultaneously have been found to be practically the same (Gilman and Cowgill).

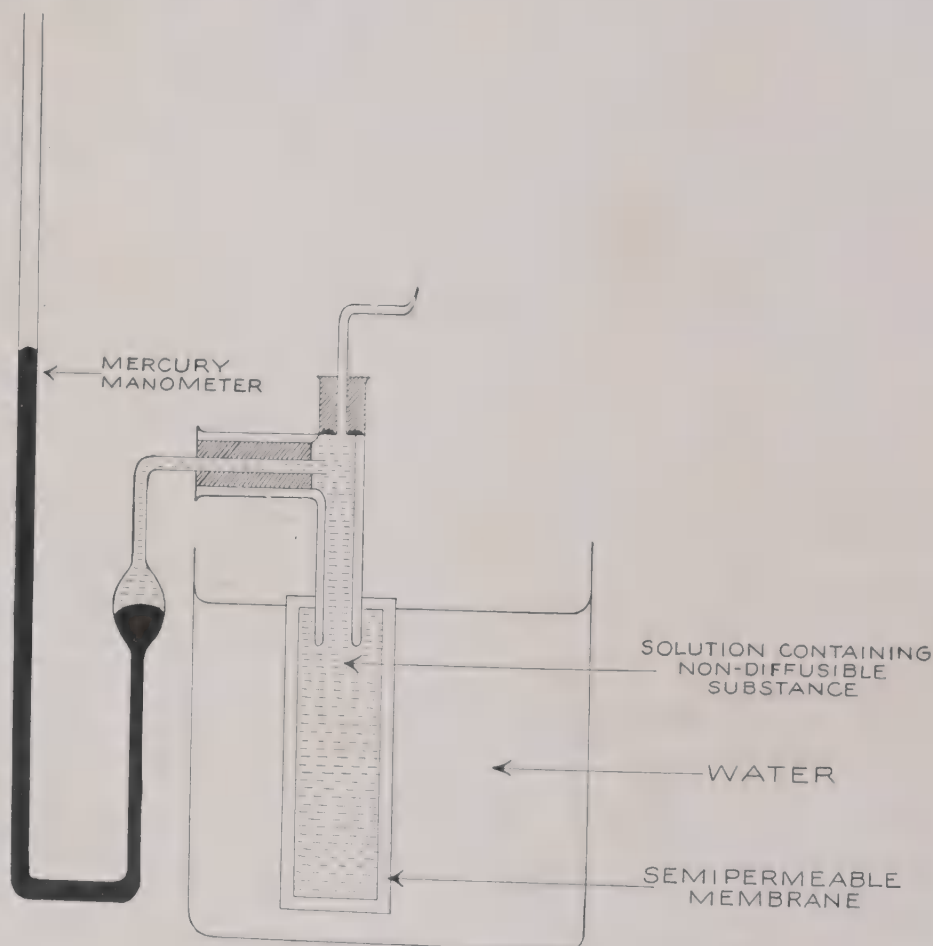


Fig. 2.—Osmometer.

**Dialysis.**—As just explained, osmosis requires a membrane which is permeable to the solvent, not to substances dissolved or dispersed in it. Membranes of varying porosity exist or can be prepared which permit particles of varying sizes to pass through but are impermeable to larger ones. In general, membranes such as parchment, collodion, or cellophane allow crystalloids to pass but prevent colloids from doing so. The process involved is termed *dialysis*. Thus, if a mixture of crystalloids and colloids is placed in a dialyzing bag which is then immersed in distilled water, the colloids will remain behind while the crystalloids



dialyze out. By changing the outside fluid frequently, the colloids may be completely freed of crystalloids. It is easily seen, however, that since colloids themselves vary in size, some membranes will permit colloids of smaller dimensions to pass.

### Membranes in the Animal Body

Since all cells—plant and animal—are enclosed by membranes, the question naturally arises: Are these membranes semipermeable or permeable, or do they behave like filter paper? This cannot be answered categorically since the wall of a living cell cannot be compared with an artificial membrane or even with a dead biological membrane. Animal membranes do not have the simple structure of artificial membranes. It is probable that cell membranes in different tissues vary in their permeability. Certainly the cells lining different parts of the kidney permit different ions and compounds to pass. Indeed, adjacent portions of the kidney tubules, for example, exhibit varying properties of this nature. It is also probable that the same cell wall will change from hour to hour, depending upon respiratory and metabolic conditions. It is therefore evident that the idea formerly held, that living membranes are impermeable to colloids but are permeable to electrolytes, must be modified.

A remarkable degree of selectivity sometimes is seen in these biological migrations. The red blood cell membrane, for example, allows almost no cations to pass through but is permeable to anions—a fact important in explaining respiratory phenomena. Furthermore, some fat-soluble as well as some water-soluble substances pass through the red cell wall. The cell membrane is believed to be a mosaic of alternate areas of lipids and proteins which are permeable, respectively, to fat-soluble and water-soluble materials. However, all the phenomena pertaining to membranes cannot be explained on the basis of porosity, i.e., sieve action, or on the theory of solubility. In some cases the electrical charge of the membrane must be taken into account. For example, if the charge on red blood cells is reversed the cells become impermeable to anions and permeable to cations.

### Gibbs-Donnan Equilibrium

If a membrane which permits the passage of crystalloids but not colloids is placed between two solutions of a simple electrolyte such as sodium chloride, the  $\text{Na}^+$  and  $\text{Cl}^-$  will pass through the membrane until, at equilibrium, the product of the concentrations of these two ions on one side equals the product of their concentrations on the other side. Concentrations such as gram molecules per liter are again represented by bracketed symbols. The conditions stated may be represented thus:

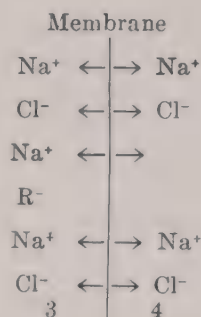
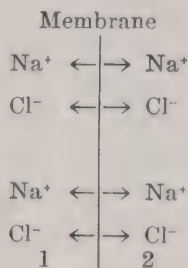
$$[\text{Na}^+]_1 \times [\text{Cl}^-]_1 = [\text{Na}^+]_2 \times [\text{Cl}^-]_2$$

$[\text{Na}^+]_1$  means, of course, the concentration of sodium ions on side 1,  $[\text{Na}^+]_2$  its concentration on side 2, etc. Moreover, not only are the products of the concentrations the same, but under these conditions the concentrations of the cations are the same, and also those of anions; that is,

$$\begin{aligned} [\text{Na}^+]_1 &= [\text{Na}^+]_2 \\ [\text{Cl}^-]_1 &= [\text{Cl}^-]_2 \end{aligned}$$



Let us assume that in addition to a simple electrolyte, we have on side 1 the sodium salt of a colloid, NaR, which can ionize to  $\text{Na}^+$  and  $\text{R}^-$  but that the colloid ion is too large to go through the membrane. Now the additional  $\text{Na}^+$  may pass through but not the  $\text{R}^-$ .



As before, chlorine ions may pass back and forth. Finally, equilibrium will occur and the question arises as to how this will affect the distribution of the diffusible ions. As in the first instance, the product of the concentrations of the diffusible cation-anion pair on one side will equal the product of the concentrations of the same pair on the other side. (This is derived from thermodynamics.)

$$(1) \quad [\text{Na}^+]_3 \times [\text{Cl}^-]_3 = [\text{Na}^+]_4 \times [\text{Cl}^-]_4$$

But

$$[\text{Na}^+]_4 = [\text{Cl}^-]_4$$

This is evident since there is only sodium chloride present on side 4 and there can be no more of one ion than the other. Now if we remember that on side 3 there is a nondiffusible ion  $\text{R}^-$  balancing electrically some of the  $\text{Na}^+$  ions, it is evident that

$$[\text{Na}^+]_3 > [\text{Cl}^-]_3$$

Now, referring to equation (1), if the members of the left side are unequal, it is clear that the larger value on the left must be larger than either of the two on the right (which are equal to each other) and the smaller value on the left must be smaller than either one on the right or:

$$\begin{aligned} [\text{Na}^+]_3 &> [\text{Na}^+]_4 \\ [\text{Cl}^-]_3 &< [\text{Cl}^-]_4 \end{aligned}$$

We can therefore see how the presence of a colloidal ion may cause an inequality in the distribution of ions on the opposite sides of a membrane. If there are several colloidal ions and many noncolloidal ions present, the state of affairs becomes exceedingly complex. This is what happens in animal tissues where different types of cells, bathed by the same or different body fluids, under varying conditions, differ fundamentally in the chemical make-up of their contents. This unequal balance of ions also leads to a difference in potential between the solutions on the two sides of the membrane. The phenomenon is known as the Gibbs-Donnan equilibrium after Willard Gibbs and F. G. Donnan who first studied and explained it.

### Viscosity

Viscosity is the resistance offered by a fluid to flow. It is due to the internal friction of the molecules of a liquid. A solvent is almost always less viscid than a solution and considerably less viscid than a colloidal system. Viscosity ordinarily is not expressed in absolute units but is referred to the viscosity of water. It is measured by allowing a definite amount of the fluid under consideration to flow through a capillary tube at a definite temperature. The time required is compared with that taken by an equal volume of water. With water as unity, the normal viscosity of blood serum is about 1.5 to 2.0, while that of plasma, which has a higher protein content, is about 20 per cent greater, and whole blood has a viscosity of 2.5 to 4.0. These are approximate values for the viscosity of normal human blood. When dehydration occurs, as it does in some pathological states, the viscosity of whole blood may be three or four times the normal value.

### Emulsions

Emulsions are dispersions of one liquid in another. If olive oil is shaken vigorously in water, it will break up into very small droplets and a yellow milky fluid will result. An emulsion is a heterogeneous system comprising two phases, but the dispersed phase consists of larger particles than colloidal particles. An emulsion of olive oil and water does not last very long; the oil droplets coalesce and soon there is a layer of oil floating on the water. Certain substances, among others, soaps, gums, proteins, when added to the system, stabilize the emulsion. It is believed that the stabilizing substance forms a protective layer around the oil droplets and so prevents them from coalescing. With any pair of nonmiscible liquids, two sets of emulsions are possible. Thus, with oil and water we may have (1) oil-in-water, and (2) water-in-oil. In a general way any emulsifying agent which stabilizes an oil-in-water emulsion will be unsatisfactory for the corresponding water-in-oil emulsion and vice versa.

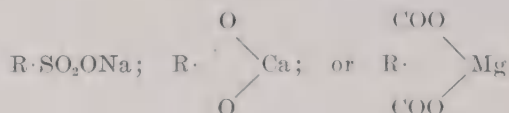
Emulsions occur widely in nature. In fact, protoplasm is probably a mixture of emulsions, containing colloids in one or both of the phases of many of them. Emulsification, of course, increases the surface of the substance emulsified. This permits biological reactions, which, as we have said, are frequently surface reactions, to take place more readily. For example, one of the functions of bile is to aid in the emulsification of fats in the small intestine. By doing so, it breaks up these water-insoluble substances into such tiny droplets that the digestive agent can very readily attack them. If this were not the case, the digestion of the mass of fat would take place extremely slowly.

### Ion Exchange Resins

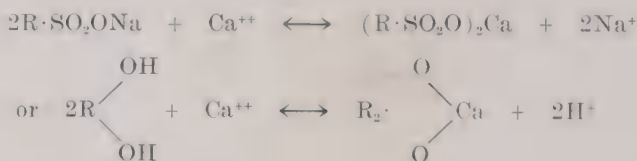
Ion exchange is a reversible interchange of ions between a liquid and a solid, involving no radical alteration in the structure of the solid. Natural products, such as certain sands, peat, and coal, operating on this principle, had been used to soften water (see page 81) for many years, but it was not until 1935 that synthetic resins were employed for this and many other pur-

poses. The English scientists Adams and Holmes reasoned that reactive acidic or basic groups which were not involved in the condensation of the constituents of resins should be free to ionize. If so, they should permit cation or anion exchange, as the case may be.

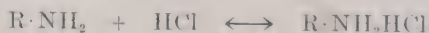
Cation exchangers are of the following types.



They exchange their cations for others and may be regenerated by treatment with a solution containing the original cation. Thus:



Anion exchangers possess activated amino groups:  $R \cdot NH_2$ . They function by a mechanism not completely understood as yet but which seems to have the effect of removing whole molecules of acid in a reversible manner. It may be compared to the reaction of an amine and an acid to form a salt.



Ion exchangers have been used in biochemistry in the analysis of amino acids and thiamine and in other ways. Clinically, they have been recommended in the determination and in the diminution of gastric acidity and in lowering the sodium content of the gastrointestinal tract in the treatment of circulatory disorders. (Segal; Spears and Pfeiffer; Kraemer and Lehman.)

### Chromatography

The discovery of chromatography is usually attributed to Tswett, a Russian botanist, in 1906, although Day, an American, had already used a column of limestone for the fractionation of crude petroleum. Tswett effected the separation of plant pigments by filtering a solution of the mixed pigments through a tube containing a finely divided solid-absorbing material. The individual pigments settled in separate bands and thus formed a *chromatogram* or pattern of pigments.

Each substance adsorbed, called an *adsorbate*, could be removed, or *eluted*, from the *adsorbent*. The term "chromatography" is still used for this type of separation, despite the fact that it is no longer limited to colored substances. It has now been greatly extended and is widely used in biochemistry as well as in other branches of chemistry.

Many different adsorbents are used and are selected for their affinity for the adsorbates. They include tale, asbestos, clays, charcoal, starches, and filter paper. Paper chromatography differs somewhat, although the principle is



the same. A large sheet of filter paper is treated in one corner with a very small amount of the solution to be studied. It is then placed over a glass rod, with the edge containing the dried solution dipping into a tray of the desired solvent. As the solvent travels up the paper, it takes with it the unknown substances, and they are deposited in spots. These spots may be "developed" as colors by suitable chemical treatment. They are identified by running controls in which known substances are used. Sometimes there is a second run, using a different solvent, at right angles, a "two-dimensional" chromatogram, which separates spots placed too closely together by the first run for easy identification.

There have been a number of explanations put forth for chromatographic phenomena, but none has been generally accepted. Therefore, many controls must be run and experimental conditions must be determined and exactly followed. This is particularly true if quantitative results are sought. (See also page 125.)

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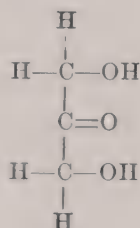
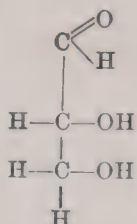


## Chapter 3

# CARBOHYDRATES

The carbohydrates include the starches, sugars, gums, and dextrins. They are found in nature and many, especially the sugars, have been produced synthetically. They are composed of carbon, hydrogen, and oxygen, the hydrogen and oxygen being present ordinarily in the same proportion as they are in water. The name carbohydrate indicates this usual composition. Examples of this molecular composition are  $C_5H_{10}O_5$ ,  $C_6H_{12}O_6$ , and  $C_{12}H_{22}O_{11}$ . Of course, compounds of quite different types have this proportional molecular constitution; e.g., acetic acid and formaldehyde. Hence, this is not a definition. A satisfactory definition of carbohydrates is: Polyhydric alcohols, having potentially active aldehyde or ketone groups, and compounds yielding them on hydrolysis. By hydrolysis is meant the breaking down of a compound by water with the addition of water, or its ions, to the products.

The simplest sugars are glyceric aldehyde and dihydroxyacetone:



Although these simplest sugars do not enter into our dietary, they are formed in the body when the more complex carbohydrates are broken down. It is evident from the definition and from these two formulas that the simple sugars are of two general types, with aldehyde and ketone groups, respectively. Since the suffix "ose" is used to designate a sugar, there are, accordingly, aldoses and ketoses.

### CLASSIFICATION

Besides the simple sugars, there are more complex ones. On hydrolysis, the complex carbohydrates yield the simple sugars. The chief classification of carbohydrates is based on this relationship:

- A. Monosaccharides, or simple sugars.
- B. Oligosaccharides, those sugars of known molecular structure, which are composed of more than one monosaccharide. The disaccharides are composed of two simple sugars. There are also trisaccharides composed of three monosaccharide molecules. Of this series, only the disaccharides are important.
- C. Polysaccharides, formed by the polymerization of a number of molecules of simple sugars to form one molecule. There are a number of different types of polysaccharides, the main ones being pentosans, hexosans, and mixed polysaccharides. The exact molecular structure of any polysaccharide is not known.

#### A. Monosaccharides

The simple sugars are further classified (1) according to whether they are aldehyde or ketone derivatives, and (2) according to the number of carbon atoms they contain. Thus there are (1) aldoses and ketoses and (2) tetroses (4C

sugars), pentoses (5C sugars), hexoses (6C sugars), etc. Combining the two, we can more accurately describe a simple sugar as a ketopentose, an aldohexose, etc. Examples are:

Trioses:	$C_3H_6O_3$ glyceraldehyde (an aldotriose) dihydroxyacetone (a ketotriose)
Tetroses:	$C_4H_8O_4$ erythrose (an aldotetrose) erythrulose (a ketotetrose)
Pentoses:	$C_5H_{10}O_5$ xylose (an aldopentose) xyloketose (a ketopentose)
Hexoses:	$C_6H_{12}O_6$ glucose (an aldohexose) fructose (a ketohexose)
Heptoses:	$C_7H_{14}O_7$ mannoheptose (an aldoheptose) mannoheptulose (a ketoheptose)

### B. Disaccharides

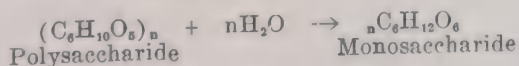
The most common disaccharides are those composed of hexoses, although theoretically any two monosaccharides could be joined to form such a compound. The most important sugars of this class are saccharose, or sucrose, lactose, and maltose. Sucrose yields one molecule of glucose and one of fructose, lactose yields glucose and galactose, while maltose gives rise to two molecules of glucose. Thus:



The disaccharides will vary not only in their constituent simple sugars, but also in the way in which these simple sugars are linked together. Indeed there may be two distinct disaccharides, with different properties, each yielding the same two monosaccharides on hydrolysis. An example of such a relationship is sucrose and turanose. Turanose has reducing powers, whereas sucrose has not. They have different melting points and are unlike in other respects as well. Yet each yields one molecule of glucose and one of fructose on hydrolysis. The reason for their dissimilarity is that the constituent monosaccharides are joined by different linkages.

### C. Polysaccharides

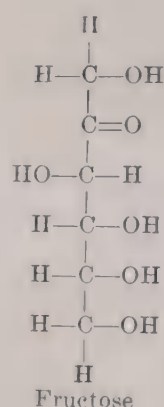
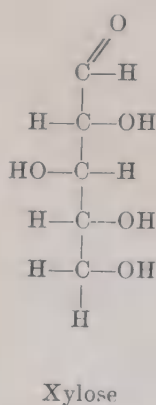
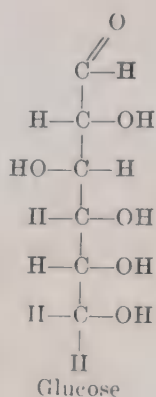
The polysaccharide group includes the starches, celluloses, and hemicelluloses. It also includes the intermediate products of hydrolysis of the higher polysaccharides, i.e., such compounds as the dextrans. There are many of these in the vegetable world, and some are to be found in animal tissues. In general they hydrolyze thus:



## STRUCTURE OF THE MONOSACCHARIDES

Most consideration will be given to the structure of glucose. This is the chief physiological sugar and will demand attention over and over again.

It is necessary to understand its chemistry and its relationship to other physiological compounds. Glucose is a monosaccharide, a hexose, and an aldose. Its molecular formula is  $C_6H_{12}O_6$ . Its structural formula may be written provisionally as shown below. Similarly xylose, an aldopentose, and fructose, a ketohexose, have the formulas shown.



However, two other physiological aldohexoses are known, namely, galactose and mannose, and still others that are not important. Similarly, a number of aldopentoses besides xylose, and ketohexoses other than fructose, are known. Glucose, galactose, and mannose all have six carbons, five hydroxyls, and an aldehyde group in their molecules. Since all differ from one another, they must have differences in the arrangement of the groups in the molecule.

### The Asymmetric Carbon

These differences in arrangement are all based on the fact that all sugars possess asymmetric carbon atoms. An asymmetric carbon is a carbon atom to which is attached *four different* atoms or groups. If a carbon atom can be

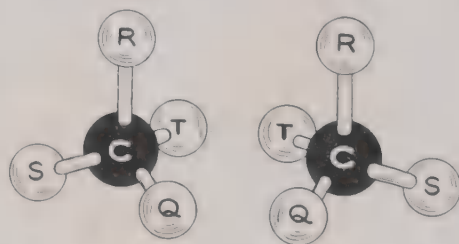


Fig. 3.—Model of an asymmetric carbon atom and its mirror image.

pictured with four bonds projected into space in four different directions (as shown in Fig. 3) and each joined to a different atom or group, Q, R, S, T, it is evident that a similar but not identical figure can be constructed, which is shown next to it. These two figures bear the same relationship to each other as that of an object to its mirror image. That they are not identical may actually be proved by attempting to superimpose one upon the other. Here are two compounds which resemble each other but are different because the carbons are asymmetric. The two hypothetical compounds shown are geometric isomers, or stereoisomers. The relationship exhibited by such compounds is called stereoisomerism; that is, their isomerism is explained on a basis of space relationships. Stereoisomers of this particular type are also called "optical"



isomers, because compounds possessing asymmetric carbons have the power of turning the plane of a beam of polarized light.

Ordinary light, vibrating in many planes, is passed through a Nicol prism in a polariscope (Fig. 4). The light which emerges is plane polarized, i.e., it vibrates in one plane. If a solution of an "optically active" compound is now placed in its path, the plane of the polarized light will be turned. To determine the direction and the degree of rotation imparted to the polarized beam, a second prism is mounted between the solution and the eye of the observer. This prism may then be rotated in the opposite direction to compensate for the twisting which the solution has produced.

Every optically active compound has a corresponding stereoisomer which rotates in an exactly opposite manner. Structurally, one is a mirror image of the other and if one rotates the plane of polarized light to the right, its mirror image will rotate it to the left. Two such stereoisomers are called antipodes.

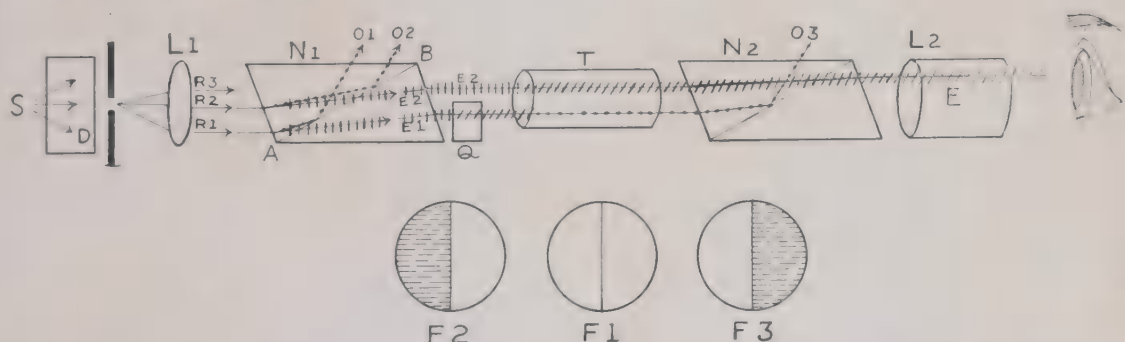
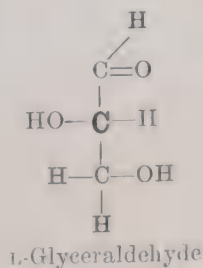
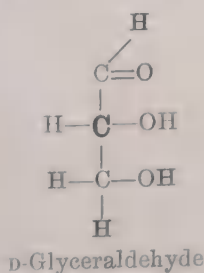


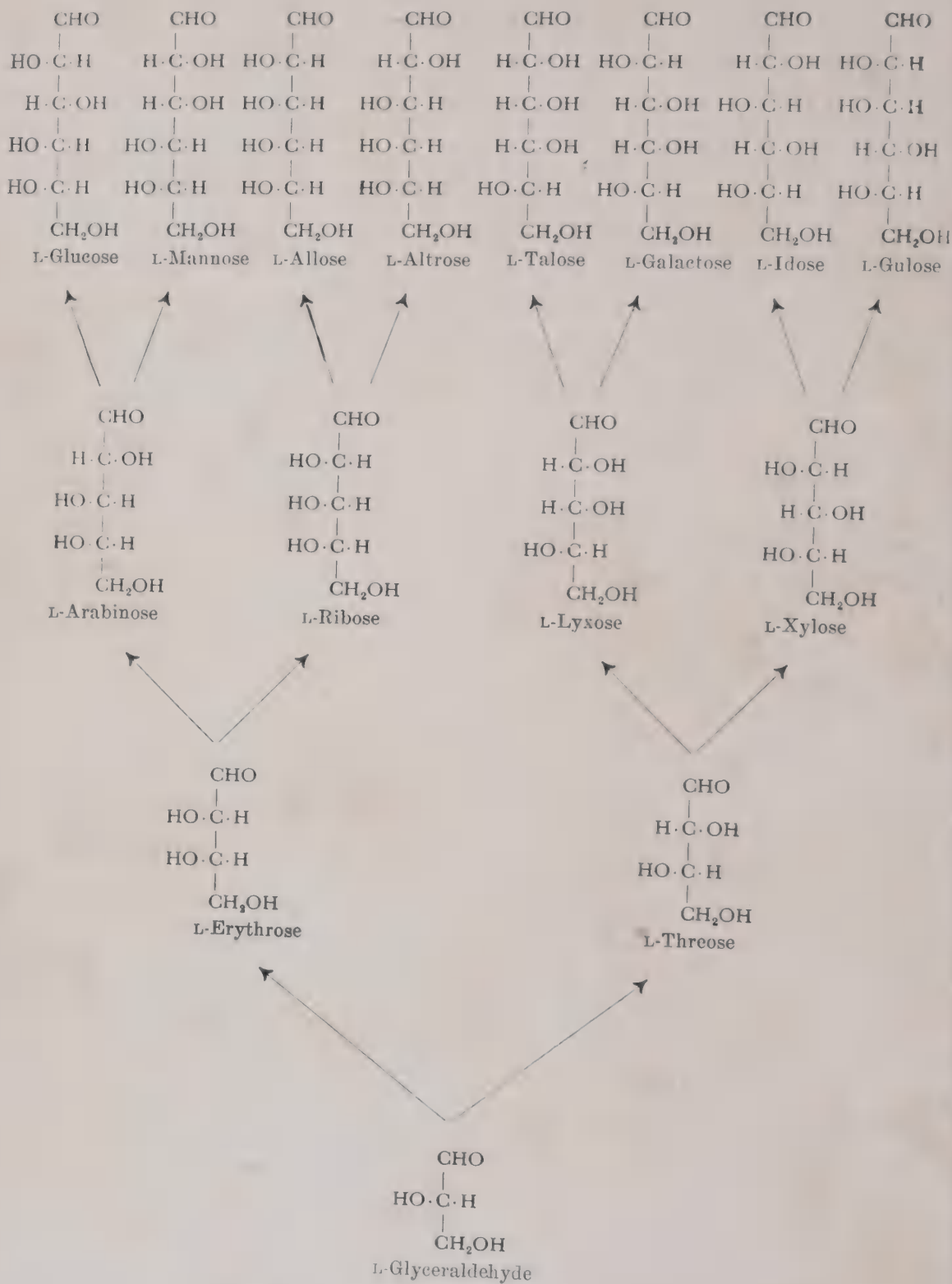
Fig. 4.—Diagram of a polariscope. *S*, Source of light; *D*, bichromate light filter, giving the effect of sodium light; *L1*, collimator lens, from which the light emerges as a parallel beam, *R1*, *R2*, *R3*; *N1*, Nicol prism, polarizer; *N2*, Nicol prism, analyzer—here it is assumed that their principal planes are parallel; *E*, eyepiece with lens *L2*.

The courses of two rays are shown. *R1* is split into two rays, vibrating in planes at right angles to each other. *O1* is the "ordinary" ray, vibrating in a plane at right angles to the page and is reflected (by the surface *AB*) and eliminated. The "extraordinary" ray, *E1*, passes out of the polarizer, vibrating in a plane parallel to the page. Similarly, *O2* is eliminated and *E2* passes out of the polarizer, vibrating in a plane parallel to that of *E1*. A quartz plate, *Q*, covering half the field, is here interposed. Actually this covers the left or right half, but in the figure it is shown as covering the lower half. *Q* turns the plane of polarized light of *E1* at a small angle to *E2*. Both now enter the tube, *T*, which contains the sugar solution. Here the planes of both *E1* and *E2* are again twisted. Now *E1* is vibrating in a plane at right angles to the plane of the page, and hence when it enters *N2* it becomes eliminated because the principal planes of *N1* and *N2* are parallel. Therefore, all the light passing through *Q* will be lost and half the field will appear dark. *E2*, although turned just as much as *E1*, is vibrating in a different plane from *E1* and, therefore, can pass through *N2* to a slight extent. The analyzer may be rotated and thus permit *E1* and *E2* to pass through to an equal extent. The field *F1* would then appear, and the amount of rotation necessary to produce this field, i.e., the compensation for the twisting due to the sugar solution in *T*, may be read off on a scale. (From Kleiner, I. S., and Dotti, L. B.: Laboratory Instructions in Biochemistry, St. Louis, 1940, The C. V. Mosby Co.)

The sugars all contain asymmetric carbons. For instance, the simplest aldehyde sugar may be represented as follows (the asymmetric carbon being shown in bold-faced type):

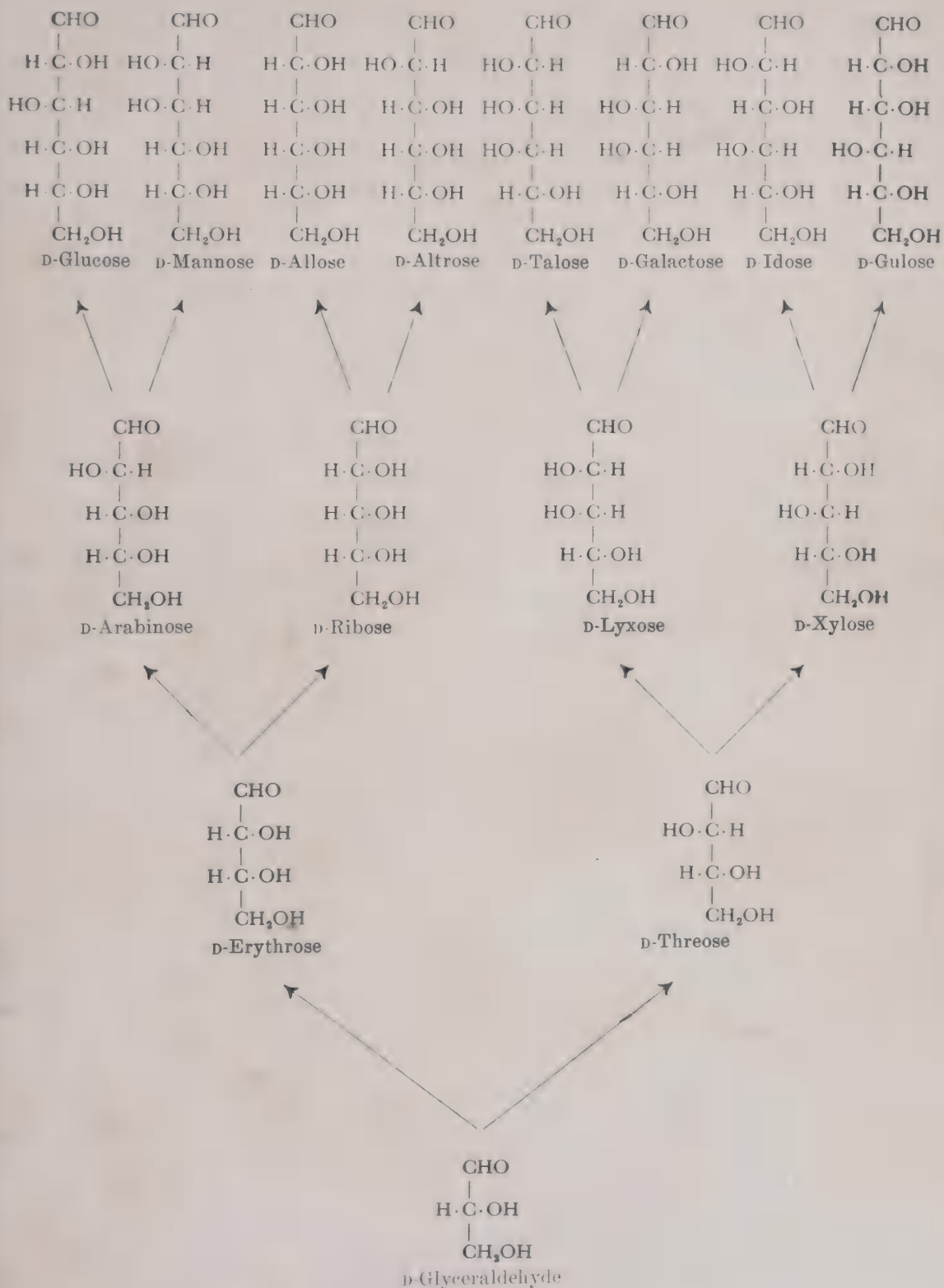






The Relationship Among the L-Aldoses

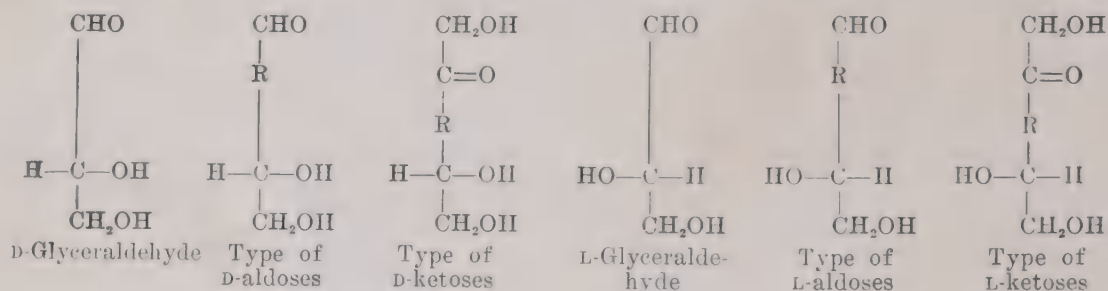
(From Karrer, P.: Organic Chemistry, New York, 1938, Nordeman Publishing Co., Inc.)



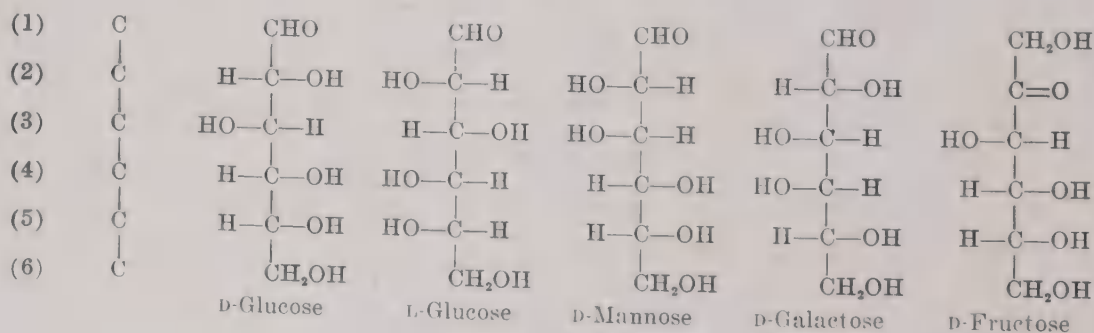
The Relationships Among the D-Aldoses

(From Karrer, P.: Organic Chemistry, New York, 1938, Nordeman Publishing Co., Inc.)

D-Glyceraldehyde rotates the plane of polarized light to the right, that is, it is dextrorotatory, while L-Glyceraldehyde is levorotatory. All simple sugars are regarded as derivatives of these two. For this reason glyceraldehyde has been called the "reference sugar." That is, all sugars which have the same configuration as D-glyceraldehyde for the corresponding two carbons are given the designation "D," regardless of whether they are dextro- or levorotatory. Those which are like L-glyceraldehyde in this respect are given the prefix "L."



Glyceraldehyde has only one asymmetric carbon atom and there are only two stereoisomers possible, and these two are known. If more than one asymmetric carbon atom is present in the molecule, the number of possible isomers is increased. Van't Hoff showed that this number would be  $2^n$ , where "n" is the number of asymmetric carbons. From the formula for an aldohexose it is evident that there are present four asymmetric carbons; accordingly there would be  $2^4$  or 16 possible isomers. Thus it can easily be seen why glucose, galactose, and mannose are all aldohexoses and are all different. It is also evident why fructose, which rotates the plane of polarized light to the left, is called D-fructose. A study of the formulas below will clarify the above statements.



### Specific Rotation

These differences in structure are responsible for variations in physical, chemical, and physiological properties of the sugars; e.g., degree of rotatory power, crystalline form, solubilities, reactions, sweetness, nutritive value. Their differences in degree of rotatory power are expressed as different "specific rotations." The specific rotation of a substance is that rotation, in angular degrees, produced by a solution containing 1 gram of substance in 1 ml. of

solution in a tube 1 dm. long. Dextrorotation is indicated by a plus sign, levo- by a minus sign. If  $[\alpha]_{\text{D}}^{20}$  is the symbol for specific rotation, using sodium light (D) at 20° C., and

$a$  = observed rotation expressed in degrees,

$l$  = length of the tube in decimeters,

$c$  = concentration in grams per 100 ml.,

then

$$[\alpha]_{\text{D}}^{20} = \frac{a \times 100}{c \times l}$$

TABLE V

VALUES OF SPECIFIC ROTATION OF VARIOUS CARBOHYDRATES AT 20° C.

(1) D-Glucose	+ 52.5	(8) Lactose	+ 55.3
(2) D-Fructose	- 92.3	(9) Sucrose	+ 66.5
(3) D-Galactose	+ 81.5	(10) Maltose	+137.0
(4) L-Arabinose	+104.5	(11) Invert sugar	- 20.6
(5) D-Mannose	+ 14.6	(12) Dextrin	+195.0
(6) D-Arabinose	-105.0	(13) Starch	+196.0
(7) D-Xylose	+ 19.0	(14) Glycogen	+197.0

By means of the polariscope, sugars may be differentiated from one another if the concentration is known. Similarly, if the sugar is known, the concentration may be determined by ascertaining the rotation, and using the equation

$$c = \frac{a \times 100}{[\alpha]_{\text{D}}^{20} \times l}$$

It should be noted that if two or more optically active substances are present in the same solution, each will exert its own rotatory power. The resulting rotation will be the algebraic sum of the individual rotations. For example, if both D-glucose and D-fructose are in the same solution, the rotation might be dextro, levo, or zero, depending upon the relative concentrations of the two sugars.

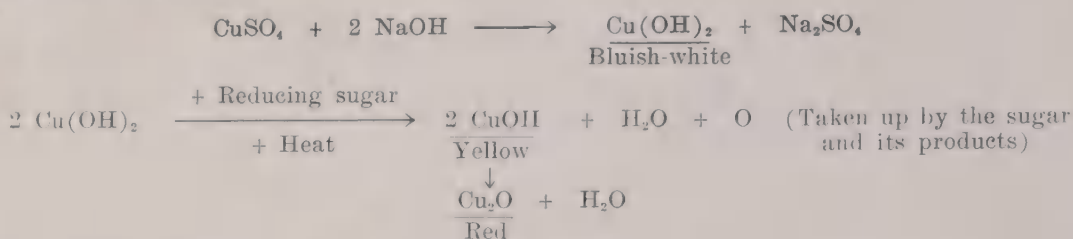
### Sugars as Reducing Agents

The monosaccharides and most of the disaccharides are rather strong reducing agents. Under suitable conditions they decompose to form fragments which reduce certain oxidizing reagents. The presence of the potential aldehyde or ketone group coincides with a great lability of the molecule to alkalis. Organic acids, together with a number of other products, result. The favorite type of reagent is an alkaline cupric solution, although other reagents both acid and basic, metallic and nonmetallic are used at times.

The fundamental reaction is shown very simply in Trommer's test. If to cupric sulfate solution is added a little sodium hydroxide, a bluish-white precipitate of cupric hydroxide is thrown down. The addition of some glucose will cause the solution of some, if not all, of this precipitate. Heating will now



lead to reduction of the cupric ions to cuprous ions and the formation of yellow to red precipitates.



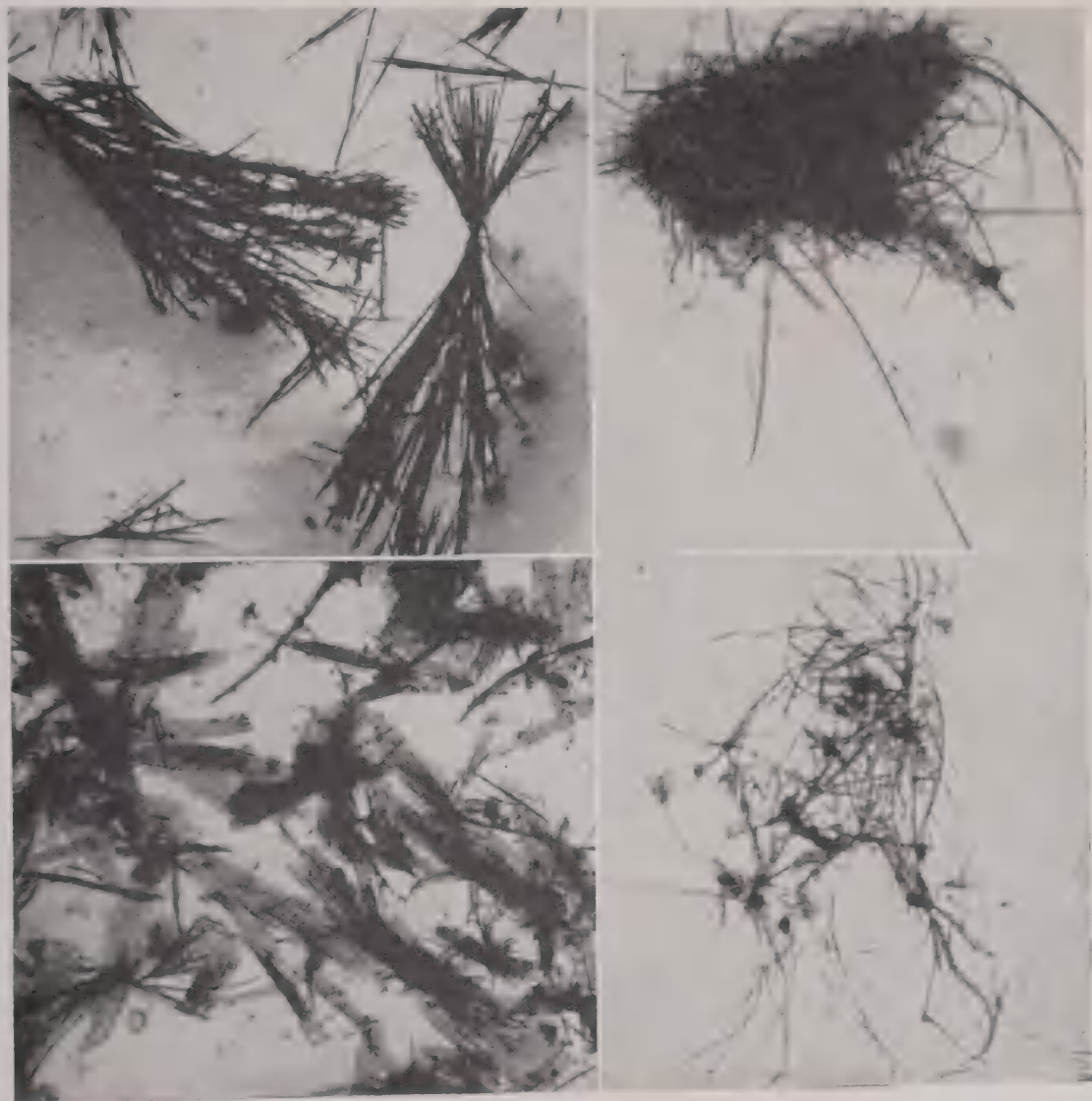
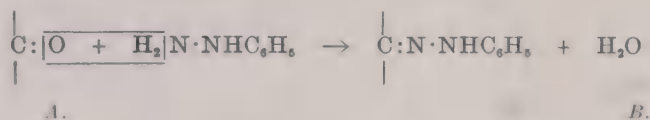
Trommer's test is not a convenient method and is seldom used. Consequently, various mixtures of reagents have been prepared which have certain advantages. An old, but still useful, reagent is Fehling's. Two solutions are needed: A is a cupric sulfate solution of definite strength, B contains sodium hydroxide and Rochelle salts (Na K tartrate). At the time of the test, equal volumes of A and B are mixed. This produces a dark blue, clear solution, since the tartrate serves to keep the  $\text{Cu(OH)}_2$  in solution. Boiling with a reducing sugar throws down a yellow to a red precipitate.

Benedict's qualitative solution is almost universally used today. This has the advantage of containing all the ingredients in a single solution. Cupric sulfate, sodium carbonate, and sodium citrate are the constituents, the citrate being the one needed to keep the  $\text{Cu(OH)}_2$  in solution. Two added advantages are: (1) the alkali is weaker and, therefore, less unpleasant to handle and (2) because of the weaker alkali, the test is more sensitive than Fehling's. Benedict's *quantitative* reagent contains, among other things, KSCN, so that a white precipitate of cuprous thiocyanate is the end product. Other alkaline metallic solutions can be used—a silver ammoniacal solution may be reduced to metallic silver, producing a mirror. An alkaline bismuth solution is known as Nylander's solution and is sometimes used clinically. Pieric acid in alkaline solution is reduced by reducing sugars to pieramic acid. This reaction is shown by a change of color from a yellowish-orange to a mahogany red. It is the basis for one of the quantitative blood sugar methods widely used for a time, and still used to some extent. In acid solution, the sugars reduce less vigorously. Barfoed's test utilizes this for distinguishing monosaccharides from reducing disaccharides. The former will react, while the latter will not. The reason for this will be seen when the disaccharides are studied. Cupric acetate in weak acetic or, better, lactic acid constitutes the reagent (Tauber and Kleiner).

### Formation of Osazones

All sugars containing a potentially free aldehyde or ketone group form *osazones* when heated with an excess of phenylhydrazine,  $\text{C}_6\text{H}_5\text{NHNH}_2$ . These are yellow crystalline compounds having characteristic forms and melting points and being considerably less soluble than the sugars from which they are derived. This is an important reaction. Practically, it is one means of differentiating the various sugars. Thus, it is an aid in distinguishing between the presence of

lactose and glucose in the urine of lactating women. Phenylhydrazine reacts with a carbonyl group to form a hydrazone:

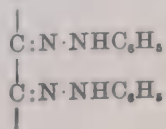


C.

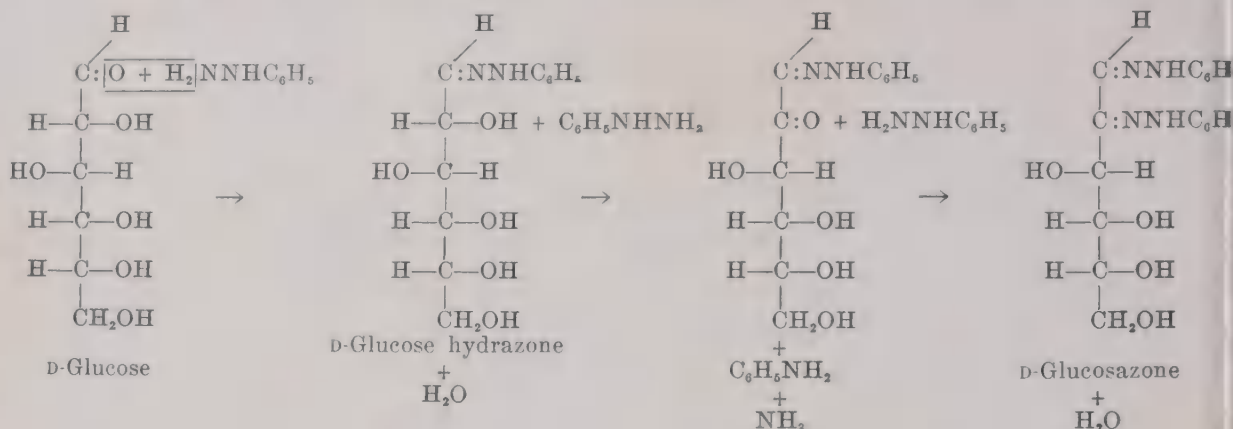
D.

Fig. 5.—Osazone crystals. A, Glucosazone; B, lactosazone; C, maltosazone; D, arabinosazone.

A second molecule of phenylhydrazine then reacts with an adjacent alcohol group, converting the latter into a new carbonyl group. Then a third molecule of phenylhydrazine reacts with the new carbonyl group in exactly the same manner as shown above, giving



which is characteristic of osazones. The reactions\* for glucose would be:



A study of the formulas on page 50 will show that D-glucose, D-mannose, and D-fructose will all yield the same osazone, because they have identical configuration for carbons numbered 3, 4, 5, and 6, whereas the other sugars shown will form other osazones. The crystalline forms of the osazones of several of the more common sugars are shown in Fig. 5.

### Desoxysugars

Sugars which have fewer oxygen than carbon atoms are known as "desoxysugars" or "desoses." Several of them are of physiological importance, particularly the pentose, 2-desoxy-D-ribose,  $\text{C}_5\text{H}_{10}\text{O}_4$ . Its formula is given on page 64. It will be apparent that, since carbon 2 is not a carbinol, this sugar cannot form an osazone.

### Action of Alkalies on Sugars

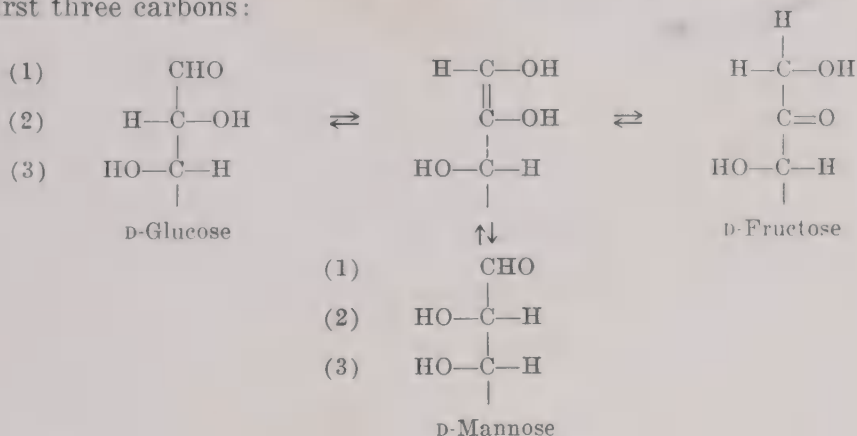
One of the reactions common to all reducing sugars is Moore's test. On warming a reducing sugar solution to which has been added a small amount of sodium or potassium hydroxide, a yellow color is seen, changing to orange and then to dark brown. A faint odor of caramel may be detected, which is intensified on acidification. Nonreducing sugars and polysaccharides do not give this test. The color is due to the polymerization of aldehydes liberated by the reaction, the final products being resins—probably similar to some of the products formed when almost any sugar is heated in the dry state to form caramel.

When treated with a weak alkali, such as barium hydroxide, glucose, fructose, or mannose is converted into a mixture of the three. Whichever sugar is used, the same proportion of all three is finally found at equilibrium. This is

\*The mechanism of formation of osazones, as formulated by Fischer, and which has been presented above, has been subject to question since phenylhydrazine does not exhibit in other reactions the function of an oxidizing agent, as it presumably does here. Two other theories (Weygand, 1940, and Fieser, 1944) have been formulated. Weygand's theory involves an intramolecular oxidation-reduction reaction resulting in the isomerism of the initially formed sugar phenylhydrazone. Fieser's theory involves the formation of tautomeric chelated ring structures. However, there is no doubt as to the number of moles of original reactants, and the formulas given above are practical for ordinary use as an identification method.



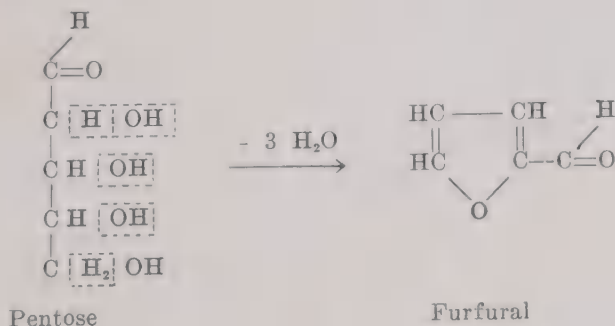
the Lobry de Bruyn transformation and is explained by the same similarity of these three sugars which accounts for their yielding the identical osazones. Remembering that carbons numbered 4, 5, and 6 have the same configuration in all three, we can show how this reaction proceeds by directing our attention to the first three carbons:



It is assumed that an intermediate compound is formed which is easily changed to any of the three sugars. Since this reaction can be easily demonstrated to occur in the test tube, it may well be the mechanism whereby one simple sugar may be changed to another in the body. We know, for example, that all utilizable sugars are eventually converted to D-glucose, and the transformation from D-glucose to D-fructose seems to be taking place continually.

### Action of Acids on Sugars

Strong acids act upon monosaccharides to yield furfural derivatives. The pentoses are converted almost quantitatively into furfural itself and this property is used in the estimation of pentoses. The reaction may be:



Since furfural and its derivatives form colored compounds with a number of organic reagents, notably with  $\alpha$ -naphthol and with thymol, the reaction can be used as a general test for carbohydrates. For example, if Molisch's reagent, an alcoholic solution of  $\alpha$ -naphthol, is added to a carbohydrate and this is treated with concentrated sulfuric acid, a reddish-violet color is produced. It should be noted, however, that substances other than carbohydrates may also be transformed into furfural derivatives and consequently give the same reaction.



## GLYCOSIDES

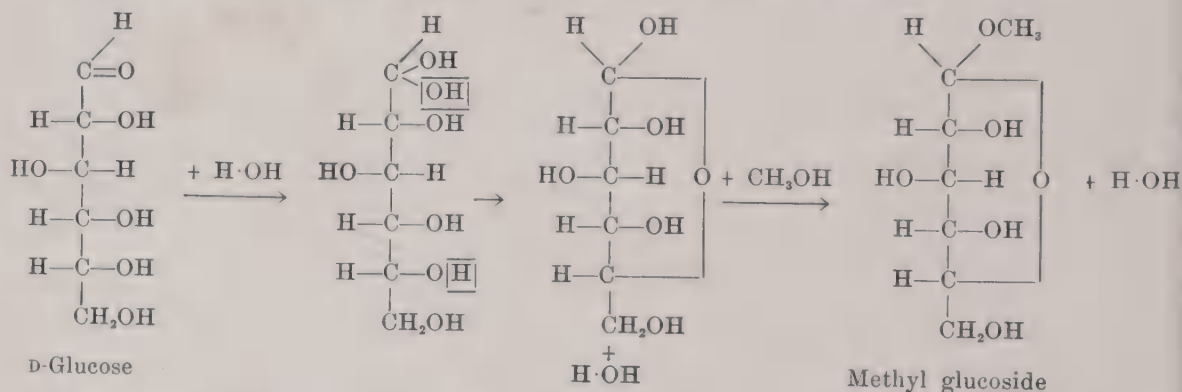
Sugars can form compounds (acetals) having an ether-like grouping of the general formula:



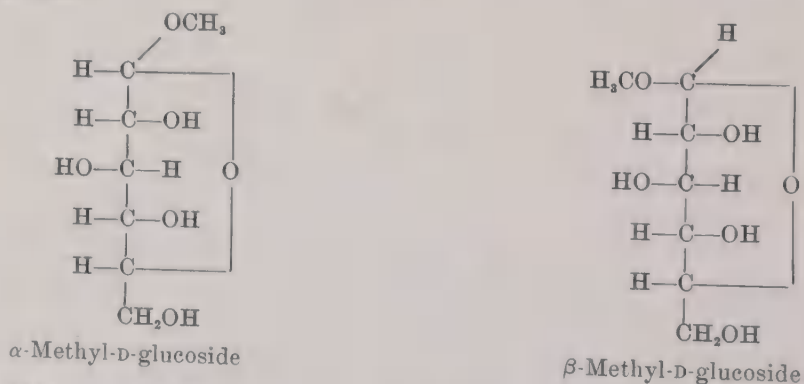
When the union is at carbon 1, the resulting compound is termed a glycoside. The R is called an "aglycone" or "aglucone" and is a nonsugar. However, the term "glycoside linkage" is used to describe the type of bridge uniting carbohydrate units with one another.

Glycosides were formerly called glucosides, but the latter term is more correctly applied to any glycoside having glucose as one of its constituents. Hence, if a glycoside has glucose as its sugar, it is both a glycoside and a glucoside. Many drugs, spices, and various constituents of animal tissues are glycosides.

If methyl alcohol and D-glucose are caused to react with each other, *two* glucosides are formed. They may be separated and purified, and it then may be shown that neither behaves as an aldehyde. The point of reaction is apparently at the aldehyde group, and the following is the probable course of events:



An examination of the formula for methyl glucoside shows that now, instead of four asymmetric carbons, there are five. The first carbon is seen to be asymmetric. Hence the two methyl glucosides, which are named alpha and beta, are represented thus:



These two differ in rotatory power, in solubilities, and in other physical characteristics. They are hydrolyzed by different enzymes—maltase acts on the alpha, and emulsin on the beta, variety.

The glycosides are very widely distributed in the vegetable kingdom and are represented in the spices, vegetable dyes, and drugs. A great number are known and more are constantly being discovered. In the plant they may have various functions, serving as reserve material, transport substances, coloring material, and poisonous and protective agents. The aglycone, or aglucone (the nonsugar residue of the glycoside), may be as simple as methyl alcohol or glycerol or as complex as the sterols. In between we have phenols, indoxyl, and the three-ringed aglucones of rhubarb, aloes, and senna.

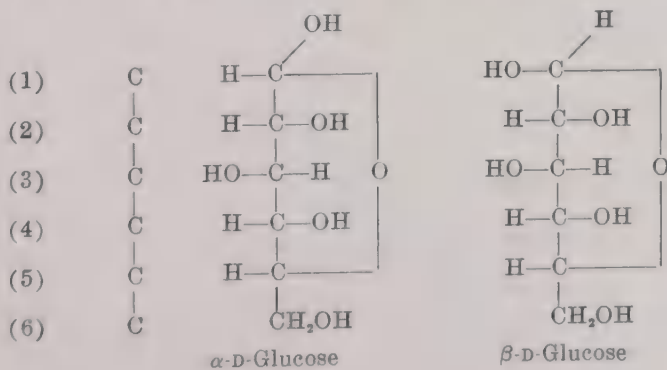
The "cardiac glycosides" are of vital importance in medicine. They are found in the leaves and seeds of *Digitalis*, the seeds of *Strophanthus*, the bulbs of squill, the flowers of lily of the valley, and in tissues of other plants. All the cardiac glycosides thus far studied are combinations of sterol hydrocarbons and a sugar molecule or chains of sugar molecules. Only a few sugars are present, namely, D-glucose, rhamnose, digitoxose, cymarose, sarmentose, and digitalose. The digitalis glycosides include digitoxin, gitoxin, gitalin, and digoxin. From *Strophanthus*, a number of glycosides have been isolated and identified by Jacobs. Among them are cymaridin, k-strophanthin-beta, and ouabain. Scillaren A is one of the glycosides of squill.

Other pharmacologically active glycosides are *phlorhizin*, which is found in the bark of the Rosaceae and has a marked influence on carbohydrate metabolism if injected into animals; *salicin*, found in willow bark; and *amygdalin*, sometimes used as an expectorant and found in the bitter almond. Among the glycosides found in spices and other plant products are *sinigrin* (in black mustard and horse-radish), *sinalbin* (in white mustard), and *saponin* (in horse chestnuts).

The union between the monosaccharide constituents of disaccharides and polysaccharides is spoken of as a glycoside linkage, even though neither member is a nonsugar.

## MUTAROTATION AND THE STRUCTURE OF THE MONOSACCHARIDES

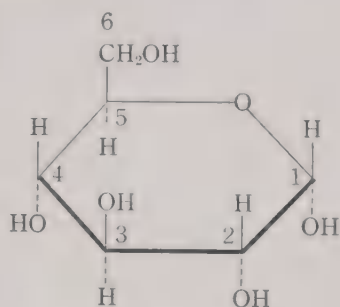
The formation of glycosides just described serves to throw light on the phenomenon of mutarotation. If some glucose is dissolved in water and observed in a polariscope, it will be found to rotate the plane of polarized light to the right, but the amount of rotation will continually change until, after some time, it becomes constant. Other sugars exhibit this same strange behavior. It is called mutarotation—changing rotation. It is explained by assuming that when the sugar is thrown into solution it is largely in one form, but after solution some of it immediately changes to another form having a different specific rotation. At a certain stage, equilibrium will be reached, and at this stage the rotation will be stabilized. Naturally, the forms of glucose to be suspected are the ones which would be supposed to form alpha and beta methyl glucoside. Therefore, we may consider D-glucose to be composed of:



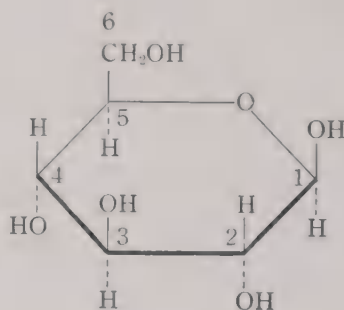
It is seen that these two formulas do not show the presence of an aldehyde radical. This harmonizes with the fact that the aldoses do not exhibit properties of true aldehydes. For example, they do not give a color with Schiff's aldehyde reagent. To account for the reducing action of sugars, one may assume that the two forms shown are in equilibrium with a very small amount of glucose in aldehyde form, and as this is used up in a reaction involving the aldehyde group, more and more is formed from the alpha and beta types.

These formulas indicate that glucose is a ring compound, a 1:5 ring as shown, although there may be 1:2, 1:3, or 1:4 rings as well. The 1:5 ring structure is also known as the "pyranose" form; that is, as a derivative of pyran. The mixture as it ordinarily occurs in solution is often referred to as "alpha beta D-glucose."

Recently the sugar formulas have been pictured in perspective. The ring of five carbons and one oxygen are shown in one plane, at right angles to the plane of the paper, with the hydrogens, hydroxyls, and one primary alcohol group either above or below the plane. The bonds below the plane are shown by dotted lines.



$\alpha$ -D-Glucose  
( $\alpha$ -D-Glucopyranose)

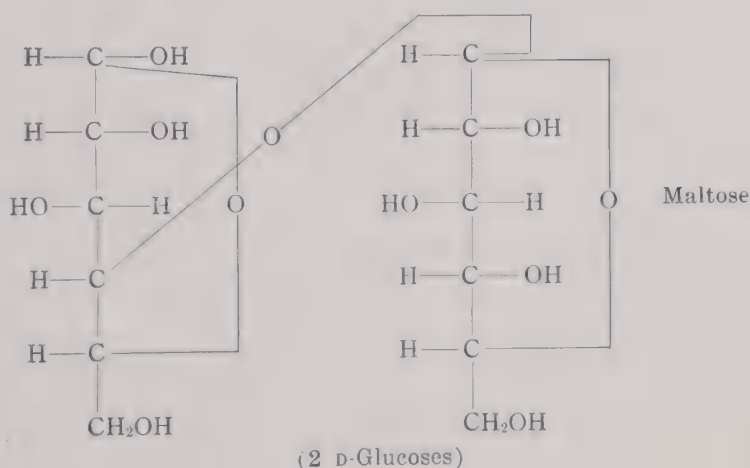
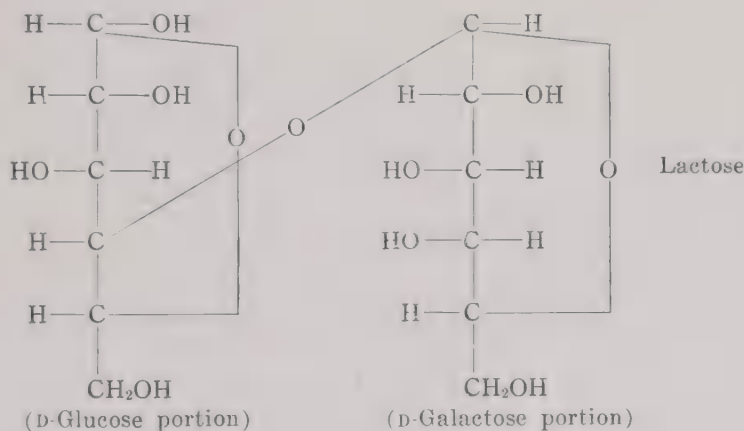
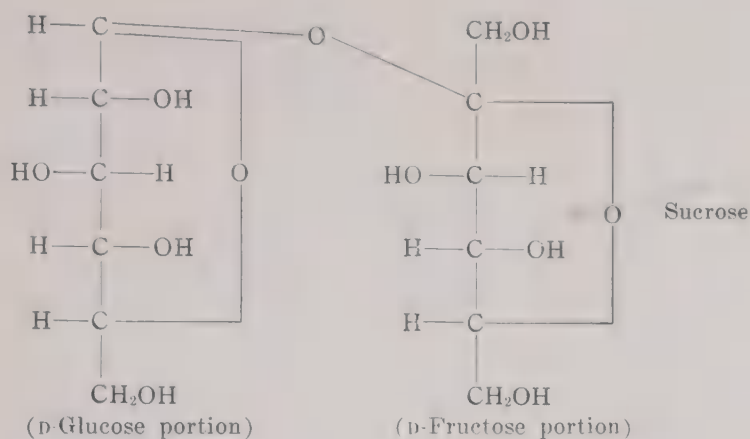


$\beta$ -D-Glucose  
( $\beta$ -D-Glucopyranose)

## STRUCTURE OF DISACCHARIDES

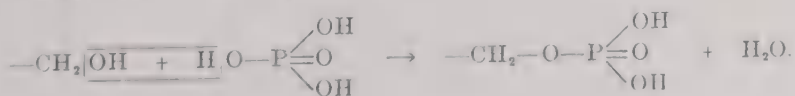
The disaccharides are formed by the union of the two constituent monosaccharides with the elimination of a molecule of water. The points of linkage vary as do the manner of linking, and the properties of the disaccharide depend to a great extent upon them. If both of the two potential aldehyde or ketone groups are involved in the linkage, the sugar will have no reducing properties and will not be able to form an osazone. However, if one of them is not bound in this way, it will permit of reduction and of osazone formation by the sugar. Sucrose is formed from D-glucose and D-fructose by union at the aldehyde and ketone carbons. It is nonreducing and does not form an osazone. Lactose and maltose both have an unlinked potential aldehyde and consequently are reducing sugars which form osazones.

A comparison of these formulas with those for alpha and beta methyl glucoside will show that maltose has an alpha glucoside linkage and lactose has a beta galactoside linkage. The constituents of sucrose are joined by an alpha glucoside beta fructoside linkage. It is also seen that in sucrose the oxygen bridge of the fructose portion is from C2 to C5. Upon hydrolysis this changes to the more stable C2 to C6 bridge.



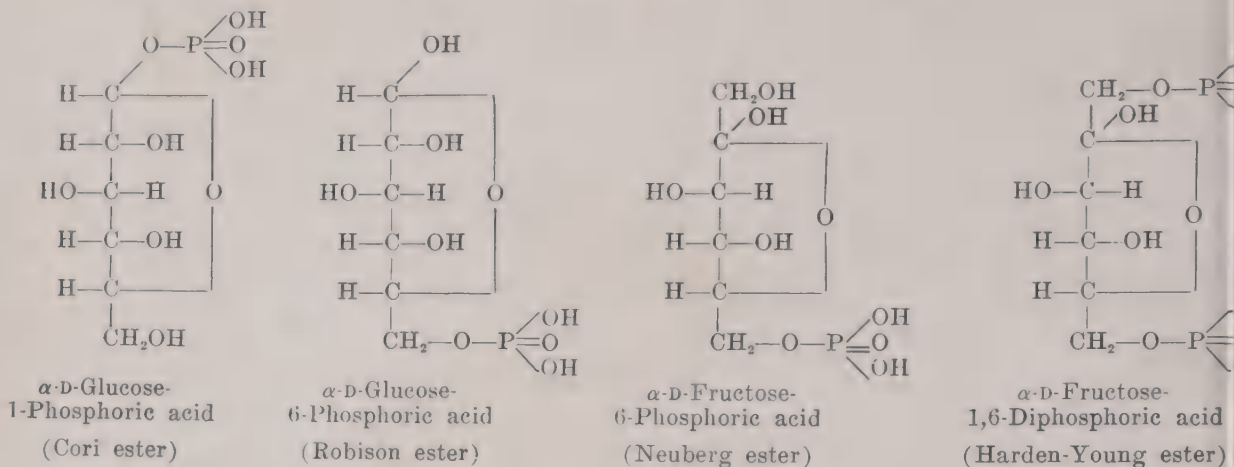
### Monosaccharide Phosphates

Esters of phosphoric acid with sugars are formed in many biological reactions. In fact, the formation of phosphates appears to be a prerequisite to most, if not all, physiological reactions. The hydroxyl radicals are esterified with phosphoric acid; thus:





Important phosphate esters of sugars include the following:

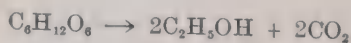


The formation of phosphates is called phosphorylation. Special enzymes and coenzymes are necessary to effect this, and these are quite specific, different enzymes being required to link the phosphate radicals to different positions. Furthermore, as glucose is broken down either in fermentation or in carbohydrate utilization in animal tissues, the fragments are phosphorylated and the phosphate group is transferred from one part of the chain to another. Finally, the end products are obtained and the phosphate radical is freed.

## FERMENTATION

When yeast is added to certain sugars, an evolution of  $\text{CO}_2$  occurs and ethyl alcohol is formed. This reaction, which has been known and utilized since pre-Biblical times, is called fermentation. A fermentation is a decomposition of an organic substance, usually a carbohydrate, produced by a living organism, or by its enzymes, with the liberation of a gas. The term "fermentation" is often loosely used for any enzymic decomposition but is more properly restricted as stated. Until Buchner's time (1897) fermentation was believed to be dependent upon the life of the yeast cells and could only be brought about by the growth, metabolism, and reproduction of such cells. That investigator, however, showed that the juice pressed out of yeast and containing no cells had the power of fermenting sugars. He called the agent present "zymase."

Ordinary baker's yeast ferments D-glucose, D-fructose, and D-mannose, as well as sucrose and maltose after hydrolyzing them, by another enzyme reaction, to their constituent monosaccharides. Other strains of yeast are known which are specific for other sugars. The reaction is commonly represented by the following equation:

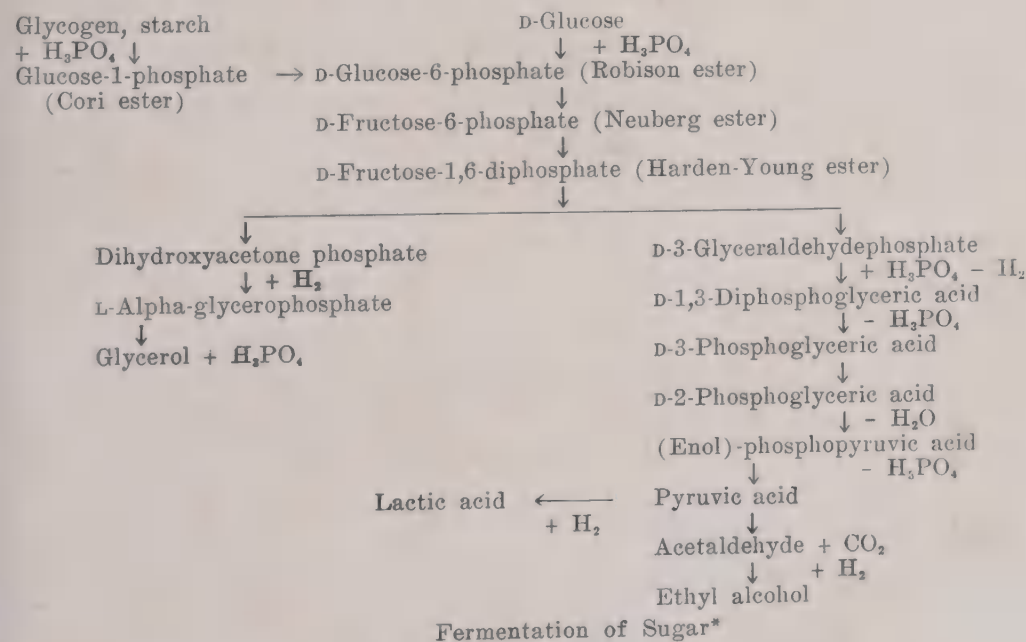


However, it is not quantitative, and products other than  $\text{CO}_2$  and  $\text{C}_2\text{H}_5\text{OH}$  are formed. Moreover, it is not a simple reaction.

It now appears that besides zymase, there are also needed a coenzyme, phosphates, and several other enzymes. We owe the explanation of this series of reactions to a number of investigators, among them Harden, Neuberg, Young, Robison, Embden, Lohmann, and Meyerhof. Dialysis of zymase solu-

tions inactivates them by removing inorganic phosphates and coenzymes, which have relatively small molecules and pass through the dialyzing membrane. Heating inactivates zymase solutions, because heating destroys enzymes. Combining the dialyzed zymase with heated zymase will restore activity. The mechanism of fermentation involves the phosphorylation of the monosaccharide, (a disaccharide is first hydrolyzed to monosaccharides by enzymic action). A series of hexosephosphates results. Then the six-carbon chain is broken to yield two three-carbon chains. A series of three-carbon phosphorylated compounds is formed, yielding, finally, several end products, including  $\text{CO}_2$  and  $\text{C}_2\text{H}_5\text{OH}$ . For these manifold transformations the presence of various enzymes, coenzymes, and inorganic ions are needed. In some instances one enzyme will catalyze more than one reaction, using different coenzymes. It is also known that oxygen modifies the rate of carbohydrate breakdown under the influence of yeast. Fermentation, with the production of alcohol, takes place optimally anaerobically, even though the yeast cells multiply more rapidly in the presence of oxygen. Smaller amounts of carbohydrates are disintegrated under aerobic than under anaerobic conditions. This is called the "Pasteur effect."

The following scheme represents Meyerhof's conception of the path of sugar fermentation. Most of the reactions indicated are reversible; i.e., they can go in both directions, depending on the concentration of the reacting products and on other factors, but they are shown here as proceeding only toward the end products.



### Monosaccharides

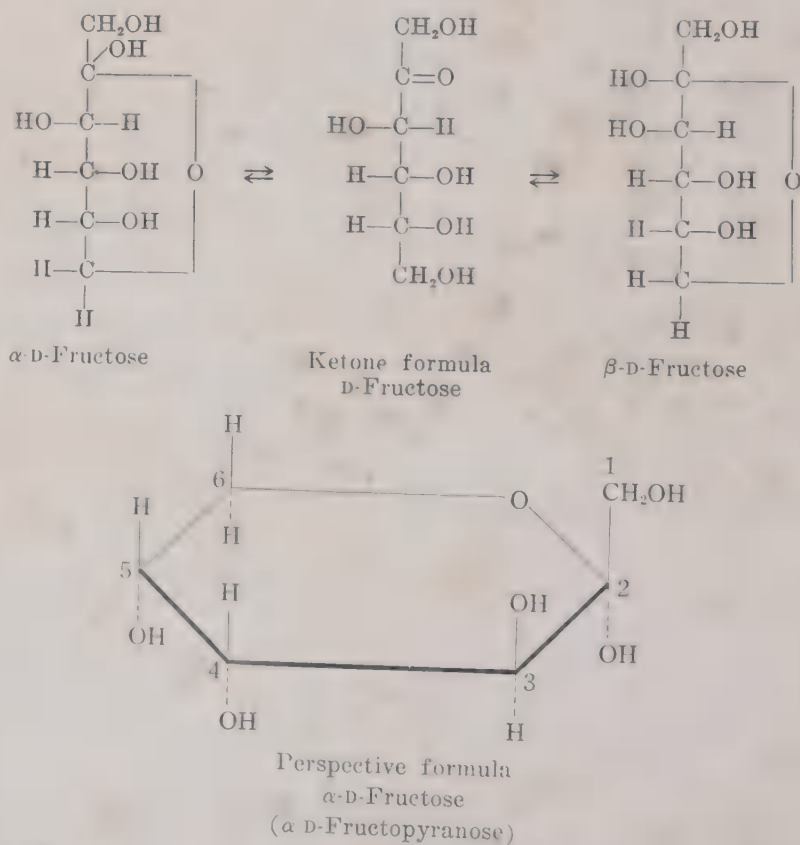
**D-Glucose.**—D-Glucose is also called dextrose, because of its dextrorotation, and grape sugar, from one of its sources. It occurs widely in fruits and vegetables, frequently associated with other sugars. Linked with a second molecule of monosaccharide, it is a component of all the common disaccharides, and in the polymerized state it is a constituent of many polysaccharides. Thus digestion

\*Adapted from Meyerhof, O.: Wallerstein Laboratories Communications 12: 255, 1949.

of these di- and polysaccharides yields D-glucose for nutritive purposes. The syrup commonly named "glucose" commercially is chiefly D-glucose. It is produced by the hydrolysis of starch. The unpleasant reputation of glucose in popular opinion is due to the fact that many years ago the sulfuric acid used in hydrolyzing grain to produce glucose sometimes contained arsenic as an impurity. The arsenic was carried over to the glucose, which resulted in some cases of poisoning. Today there is no possibility of such an occurrence. Consequently there is no ground for prejudice against glucose syrups for use in the dietary. These syrups, the so-called corn syrups, contain some dextrans and maltose besides glucose and are used to some extent to modify cow's milk for use in infant feeding.

D-Glucose is the physiological sugar and, probably, any other sugar, which has nutritive value, must first be converted to that form. Later it may be transformed into other monosaccharides for special uses. It is present in normal blood continually and at a fairly constant level, i.e., about 0.1 per cent. There are also minute traces normally in the urine, but these are too small to be detected by ordinary methods. Pathologically both blood sugar and urine sugar may increase considerably. The disease *diabetes mellitus* is a notable example.

**D-Fructose.**—D-Fructose is a keto-hexose. Its molecular formula is  $C_6H_{12}O_6$ , and its structural formula may be shown in three ways: (1) as a ketone (D-fructose), (2) as alpha and beta D-fructose, and (3) in the perspective



formula. It should be noted that the extra asymmetric carbon to account for mutarotation, etc., is carbon 2 in the case of fructose. Fructose is commonly



called fruit sugar because of its widespread occurrence free in fruits. It is one of the constituents of sucrose and of other sugars, and also of the polysaccharide inulin. It is a very sweet sugar, much sweeter than sucrose, and more reactive than glucose. It rotates the plane of polarized light to the left, whence another name is derived, namely, *levulose*. It also exhibits mutarotation. The letter *D*, which is prefixed in its exact scientific name, *D*-fructose, of course, refers to its configuration. That is, in the ketone formula it has the same configuration for carbons 4, 5, and 6 as has *D*-glyceraldehyde, the reference sugar. Since carbons 3, 4, 5, and 6 have the same arrangement of H's and OH's as those of *D*-glucose, the osazones of these two sugars are identical.

Fructose can further be distinguished from glucose by Seliwanoff's reaction. In this procedure a few drops of dilute sugar solution are heated with a reagent containing resorcinol in strong HCl under carefully regulated conditions. There is formed hydroxymethylfurfural which reacts with the resorcinol to give a red compound. Other ketoses and ketose-containing disaccharides, such as sucrose, will give this test, and other sugars will also, if present in high concentrations or if the heating is continued beyond the proper period. A more decisive reaction is the formation of the methylphenylfructosazone. This is specific for fructose and can be carried out in the presence of glucose or sucrose (Neuberg and Mandl).

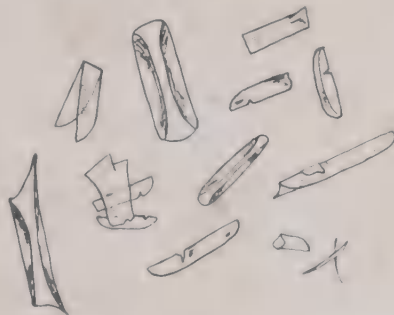


Fig. 6.—Mucic acid crystals.

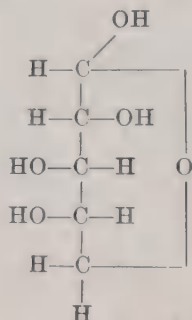
**D-Galactose.**—*D*-Galactose is seldom found free in nature, but in combination it occurs both in plants and animals. In plants it appears in certain polysaccharides (for example, in the seed coats of the legumes) and in complex mixed carbohydrates. Indeed it is claimed that carbohydrates yielding galactose are almost as widespread in plant seeds as is sucrose. It apparently can be manufactured by the body or, more probably, glucose is changed into galactose. Among other sites of this transformation is the mammary gland, for milk sugar is made up of glucose and galactose. It is also a constituent of the glycolipids which are found in many tissues, but particularly in nervous tissue. Pathologically, it occurs in the urine of nursing infants having digestive difficulties.

Galactose is not as sweet as glucose and is less soluble in water but has a higher specific dextrorotation. On oxidation it yields mucic acid which is less soluble than the corresponding acid formed from glucose. This aids in its identification, since the crystals of mucic acid are not difficult to produce and to detect (Fig. 6). Another difference is that it is fermented much more slowly by yeasts than is glucose. Bakers' and brewers' yeast do not ferment it at all.

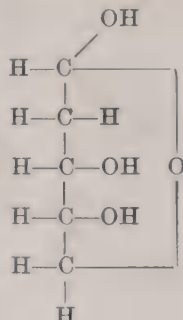


**D-Mannose.**—D-Mannose is not found free to any great extent, and the polysaccharides which yield it on hydrolysis are not very digestible. It is, therefore, of very slight importance nutritionally. Bakers' yeast ferments it.

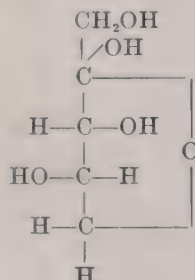
**Pentoses.**—The most important pentoses—all having the molecular formula  $C_5H_{10}O_5$ —are L-arabinose, D-xylose, D-ribose, L-xyloketose, and also 2-desoxy-D-ribose ( $C_5H_{10}O_4$ ). The first four are aldoses and are not found free in nature. Arabinose results when arabans are hydrolyzed. Gum arabic, cherry gum, and other gums contain arabans. D-Xylose is produced by the hydrolysis of the xylans of straw and wood. D-Ribose and 2-desoxy-D-ribose are constituents of nucleic acids (acids which are found in combination in all cells). Desoxy sugars contain one oxygen atom less than the sugars from which they are derived. The last of the five pentoses mentioned, L-xyloketose, is, according to Greenwald, the sugar present in the urine in the rather uncommon condition known as pentosuria. The structure of three of these is shown, all in the alpha forms.



L-Arabinose



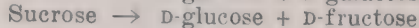
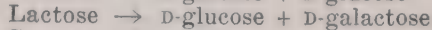
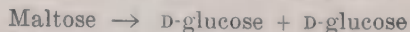
2-Desoxy-D-ribose



L-Xyloketose

### Disaccharides

The three most common disaccharides, maltose, lactose, and sucrose, have the molecular formula  $C_{12}H_{22}O_{11}$  and hydrolyze according to the following equation:



**Maltose.**—Maltose, or malt sugar, is an intermediary in the acid hydrolysis of starch and can also be obtained in enzyme hydrolysis of starch. One of the time-honored methods utilizes the action of sprouting barley, which contains the enzyme diastase, upon grain. This is the first step in the production of alcohol. Various food preparations, such as baby and invalid foods, produced by hydrolysis of grains, contain large amounts of maltose. Starch digestion in the body yields maltose, which only requires one further hydrolytic action to be converted into glucose. It is a rather sweet sugar and is very soluble in water. Since one aldehyde is free, or potentially free, it has reducing properties and forms a characteristic osazone.

**Lactose.**—Lactose is milk sugar. It is found only in milk in appreciable quantities. It is, to be sure, found also in very low concentration in the blood and urine of lactating women, but this should be considered accidental and is by no means a constant occurrence. If the mammary gland produces an excess

of lactose, or if the milk is not removed rapidly enough, lactose will dam back into the circulation. The body cannot utilize unhydrolyzed disaccharides; therefore this "wandering" lactose will be excreted by the kidneys. Lactose also has reducing properties and forms a characteristic osazone. It is not very soluble and is not as sweet as the other common sugars. This property may be made use of when it is desirable to force carbohydrate feeding in patients having a distaste for sweets. It is dextrorotatory. Because it contains galactose as one of its constituents, it yields mucic acid upon oxidation. It is not fermented by baker's yeast and its behavior toward other microorganisms is also noteworthy. It is fermented by the colon bacillus but not by the typhoid. This reaction is used by bacteriologists to distinguish between these two organisms, which resemble each other in many ways. Many organisms which are found in milk; e.g., *Bacillus coli*, *Bacillus aerogenes*, and *Streptococcus lacticus*, convert lactose into lactic acid, thus causing the souring of milk. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* are examples of other organisms, not ordinarily found in milk, which have the same action; these two are used to produce the therapeutic sour milks.

**Sucrose.**—Ordinary table sugar is sucrose, or saccharose. It is obtained from the sugar cane, the sugar beet, and, to a lesser extent, from the sugar maple. It also occurs in a number of fruits and vegetables; e.g., pineapples and carrots. It is very soluble, very sweet, and on hydrolysis yields glucose and fructose. Sucrose is dextrorotatory, but its hydrolysis products are levorotatory because fructose has a greater specific levorotation than the dextrorotation of glucose. Since this hydrolysis therefore inverts the rotation, the mixture of glucose and fructose is called "invert sugar" and the process is called "inversion." Honey is largely invert sugar and the presence of fructose accounts for the greater sweetness of honey. Sucrose does not reduce alkaline copper solutions, nor does it form osazones. The explanation of this is that the aldehyde and ketone groups of its constituent monosaccharides are linked together and consequently neither one is free to act. Sucrose is fermented by ordinary yeast but is first inverted by invertases present in, or secreted by, the yeast cell. Sucrose is easily inverted by invertases present in the intestinal tract and is the most important sugar of our dietary. The monosaccharides formed—glucose and fructose—are readily absorbed and utilized by the body. It must be emphasized, however, that this is only true of sucrose taken into the body per os. If introduced by any path other than the gastroenteric tract, i.e., *parenterally*, it is hardly utilized at all. Disaccharides are unphysiological substances when present in the blood stream, as previously mentioned in the case of lactose. Therefore, sucrose should not be injected intravenously if its nutritive properties are desired. It sometimes is injected in this way, but the clinician uses it to increase the osmotic pressure of the blood and to cause a flow of water from the tissues into the blood. Invert sugar, however, may be given intravenously as a nutrient fluid.

### Sweetness of Sugars

The relative sweetness of sugars cannot, of course, be determined with great accuracy. However, observations on a series of subjects gave the figures shown

in Table VI. Sucrose was used as the standard and the relationship of this sugar to the other sugars was determined by observing the highest dilution at which the sweet taste was detectable.

TABLE VI  
RELATIVE SWEETNESS OF SUGARS\*

SUGAR	NUMERICAL RATING (SUCROSE = 100)	UNITS OF WEIGHT OF SUGAR EQUIVALENT TO ONE UNIT OF SUCROSE
Lactose	16.0	6.3
Raffinose	22.6	4.4
Galactose	32.1	3.1
Rhamnose	32.5	3.1
Maltose	32.5 (†)	3.1 (†)
Xylose	40.0	2.5
Glucose	74.3	1.3
Sucrose	100.0	1.0
Invert sugar†	127.4	0.8
Invert sugar‡	130.0	0.8
Fructose	173.3	0.6

\*Data from Biester, A., Wood, M. W., and Wahlin, C. S.: Am. J. Physiol. 73: 387, 1925; and Willamen, J. J., Wahlin, C. S., and Biester, A.: Am. J. Physiol. 73: 397, 1925.

†Prepared by the action of invertase upon sucrose.

‡Equal parts of glucose and fructose mixed.

### Polysaccharides

The polysaccharides are much more complex substances than the other carbohydrates so far discussed. Most of them are white amorphous compounds, of which starch is a typical example. They are not sweet and, when pure, do not reduce or give other characteristic aldose or ketose reactions. Since they are polymers of sugars, the size of the polysaccharide molecule is, in general, very large. Consequently, those that are not insoluble form colloidal solutions. The molecular weights of the celluloses probably range from 200,000 to 400,000; those of the starches, from 10,000 to 1,000,000; and glycogen from different sources is believed to vary from 1,000,000 to 4,000,000.

The polysaccharides are classified according to the type or individual monosaccharide yielded on hydrolysis. Thus starch belongs to the general group of *hexosans*, because a hexose is the product of its hydrolytic decomposition. It is, more particularly, a *glucosan* since glucose is the particular hexose involved. There are three main groups:

- A. Pentosans, yielding pentoses; xylans and arabans are subgroups, yielding xylose and arabinose, respectively.
- B. Hexosans, yielding hexoses; glucosans, mannans, galactans, and fructosans yield the sugars indicated by their names.
- C. Mixed polysaccharides; complex polysaccharides yielding, on hydrolysis, more than one kind of sugar, and frequently sugar derivatives.

**Pentosans.**—Pentosans occur chiefly in vegetable gums, such as cherry gum, and in other vegetable materials, such as straw. On hydrolysis arabans yield L-arabinose, and xylans, D-xylose. Little is known of their role in plant and animal physiology. Gum arabic is not the pure pentosan it was formerly thought to be. It probably contains a considerable amount of pentosan since mild treatment with acids releases arabinose, but there is left behind a polysaccharide nucleus resistant to acid. This nucleus is a mixed polysaccharide.



**Hexosans.—**

**STARCH.**—Starch occurs in many plants as storage foods. It may be found in the leaves and stems as well as in the roots, fruits, and seeds where it is usually present in greater concentration. Starch granules under the microscope appear as particles made up of concentric layers of material. They differ in shape, size, and markings with the plant (see Fig. 7). In this way experts can identify starches which have been mixed with spices or other products as adulterants. Starchy foods are the mainstay of our diet. Large amounts are present in cereals such as wheat, rye, corn, and barley, in potatoes, in legumes, and in nuts. In the ripening process in some plants, such as the apple and banana, starch is changed to sugar; in others, as for example corn and peas, the change is in the opposite direction. Starches also are used in industry and in the household in many ways, as, for example, in laundering.



Fig. 7.—Starch granules ( $\times 200$ ). (From Fearon, W. R.: *An Introduction to Biochemistry*, ed. 2, St. Louis, 1940, The C. V. Mosby Co.)

The starch granule contains two hexosans, both polymers of glucose but differing in molecular architecture and in certain properties. One is called “amylose” and the other “amylopectin.” As present in the starch granule, amylose is more soluble in hot water than is amylopectin. On drying, however, the amylose becomes more and more insoluble. Starch forms a colloidal solution when boiled with water, “starch paste.” In this the amylopectin is believed to act as a protective colloid for the amylose. Both the starch grains and the colloidal solution give an intense blue color when treated with iodine. The intensity of the color produced by amylose is about six times that produced by amylopectin.

Hydrolysis of starch yields a succession of polysaccharides of gradually diminishing molecular size, while maltose is simultaneously split off.

Iodine Reaction	Course of Hydrolysis	
Blue	Starch	
Blue	Soluble starch	Maltose
Purple	Amylodextrin	Maltose
Red	Erythrodextrin	Maltose
Colorless	Achroödextrin	Maltose
	Maltose	

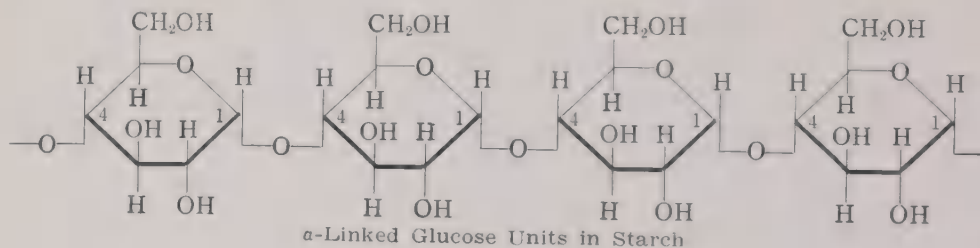
Enzyme hydrolysis (amylase) ends at maltose and, of course, is not quantitative; traces of the dextrans remain. If a maltase is present, or if the hy-



drolisis is accomplished by acid, much of the maltose will be converted into glucose.

Starches are capable of forming esters with either organic or inorganic acids. There is present in some starches a minute amount of phosphoric acid. This is linked by primary ester bonds to carbon 6. Some synthetic starch esters are useful as plastics, while starch nitrates are violent explosives.

The exact molecular structure of both amylose and amylopectin is not entirely settled, although the general pattern of each is known. Amylose consists of long chains of from 70 to 2,100 glucose units (Meyer and Rathgeb; Hassid). Haworth's suggestion is a chain structure of glucose units joined together by  $\alpha$ -glycoside linkages from the first carbon atom of one glucose unit to the fourth carbon of the next one. Since the aldehyde-bearing carbon (C1) is joined in each case to the next unit, its reducing action is nullified. A portion of such a structure is shown thus:



The amylopectin molecule is thought to be made up of a large number of branches, each consisting of 25 glucose units or less. Each of these small branches resembles the larger amylose chains but they are joined together in such a way that the free-reducing group of a glucose unit at the end of one branch is glucosidically linked through the sixth carbon of a glucose unit (not an end one) in an adjoining chain. The resulting molecule is much larger than that of amylose. (See Fig. 8.) There is at present no agreement as regards the pattern or mode of branching.

**DEXTRINS.**—When starch is hydrolyzed by the action of acids or enzymes, it is broken down into a number of products of lower molecular weight known as dextrins. These include “soluble starch” and the other dextrins listed on page 67. “Soluble starch” forms a clear colorless solution, not at all “starchy” in appearance but giving a blue iodine reaction. The other dextrins are water-soluble and react to iodine as indicated above. They resemble starch by being precipitable by alcohol, forming sticky, gummy masses. Dextrin solutions are often used as mucilages; the mucilage on the back of the postage stamp is an example. Starch hydrolysates, consisting largely of dextrins and maltose, are widely used in infant feeding. These carbohydrates are not only easily digested, but their physical properties are useful in preventing the formation of large heavy curds when the milk, with which they are mixed, clots in the baby's stomach. Many breakfast foods contain dextrins, as do the malt preparations used in soda fountain beverages. Most preparations of dextrins are mixtures. One well-defined dextrin is called “limit dextrin.” This is the product remaining after the enzyme  $\beta$ -amylase has acted upon starch until no further action is observed (Meyer and Gibbons).

**DEXTRANS.**—Certain microorganisms produce polysaccharides known as dextrans, when grown on sugar media. These constitute what was formerly called “slime” and were discarded when they appeared in industrial operations. They differ from dextrans in structure. They are made up of units of a number of D-glucose molecules having  $\alpha$ -1,6 glycosidic linkages within each unit, and the units are joined together to form a network. Dextrans, synthesized by *Leuconostoc mesenteroides* in a pentose medium, have recently been recommended as blood “extenders”; that is, solutions of them may be injected intravenously, after blood loss, in order to increase the volume of the circulating blood. (See page 198.) Because of their high viscosity, low osmotic pressure, and the slow disintegration and utilization of the dextrans, they remain in the blood for many hours. If the injections are not repeated too frequently, the dextrans are eliminated and do very little damage to the tissues. (Grönwall and Ingelman.)

**GLYCOGEN.**—Glycogen is often called animal starch, although now it is known to occur also in yeasts and in algae and fungi and a polysaccharide very similar to it is in golden bantam sweet corn. It is found in large amounts in oysters and other shell fish and the muscle of the scallop is particularly rich in it. In higher animals it is deposited in the liver as storage material and in the muscle as a more immediate source of energy.

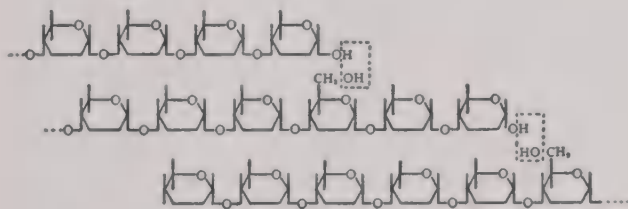
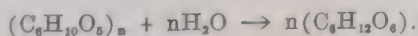


Fig. 8.—Haworth and Hirst's diagrammatic formula for amylopectin. Each chain (repeating unit) contains about 25 glucose residues.

It may be precipitated from its beautifully opalescent solution by ethyl alcohol and, in drying, forms a pure white powder. It is dextrorotatory with an  $[\alpha]_D^{20} = 196^\circ.6$ . Glycogen is not destroyed by a hot strong KOH or NaOH solution. This property is made use of in the method for determining it quantitatively in tissues. The weighed minced tissue, to which strong alkali has been added, is heated on a steam bath until the tissue disintegrates and dissolves; then the glycogen is precipitated out by alcohol and, after purification, it is hydrolyzed to glucose, which may be determined by some standard quantitative procedure. With iodine, glycogen gives a deep red color. In this respect it resembles erythrodextrin, but it may be distinguished from this substance by its opalescence in solution and by certain other properties. The glycogen-iodine reaction requires a much stronger iodine reagent than does the starch-, or dextrin-iodine test.

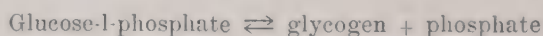
Glycogen on hydrolysis yields D-glucose:



An analagous reaction is constantly occurring in the body. However, in the body it is a reversible reaction and phosphoric acid is involved (see Chapter 16).

Glucose, and other monosaccharides, are transformed into glycogen in muscle and liver, and glycogen is rapidly disintegrated to furnish D-glucose for physiological requirements as needed. Glycogen resembles amylopectin in structure in that it is made up of branched chains of glucose units. The individual glucose units are attached to each other by the same  $\alpha$ -1,4-glucosidic linkages but the chains are somewhat shorter, averaging 12 glucose units. These short chains are joined together in much the same way as are those of amylopectin. Glycogen, as well as many other polysaccharides, does not exist in the form of homogenous molecules all having the same molecular weight and possessing the same numbers of monosaccharide units. The glycogen units are collections of polymers, having the same general branching plan, but a rather wide range of molecular weights. (Bell.)

Lazarow has isolated from guinea pig livers, by high speed centrifugation, a glycogen complex which is termed "particulate glycogen." This contains a high percentage of water and the dry matter is composed of about 93 per cent glycogen and 1 per cent protein. It is believed that protein may combine with glycogen, thus removing it from the reaction mixture and shifting the reversible reaction



toward the right and facilitating glycogen storage. (See Chapter 16.) The high content of water also probably has some physiological significance.

**CELLULOSE.**—Cellulose is a hexosan which makes up a large part of the framework of plant tissues. It is quite insoluble, except in certain special reagents. Absorbent cotton and filter paper are composed very largely of cellulose. It is not hydrolyzed readily by dilute acids but heating with fairly high concentrations of acids yields the disaccharide cellobiose. Cellobiose is made up of two molecules of D-glucose, linked together by  $\beta$ -glucosidic linkages between the first and the fourth carbon atoms of adjacent glucose units. Hence cellulose is considered to be long chains of glucose units joined together in this way. No cellulose-splitting enzyme is secreted by the mucosa of the human gastrointestinal tract. However, some microorganisms are capable of digesting it and if such are present in our intestinal canal there is the possibility of some slight nutritive value. Even such forms as termites, which use wood and other cellulose-containing materials as food, do not do so directly. Cleveland has shown that there is a symbiosis between these insects and their intestinal protozoa. The teeming protozoa either digest the cellulose completely or else play a very important part in its digestion. Although cellulose cannot be considered of any particular value nutritionally, it does have a physiological role. It is a part of the "roughage" or indigestible matter of the diet. This is swept along the gut by the intestinal peristaltic wave and the mere bulk is of value in stimulating this movement. In the large intestine its bulk aids in the formation of normal stools. Often an increase in roughage will relieve constipation, but too great an amount is not desirable and is even likely to aggravate the condition.

Methyl cellulose has also been suggested for certain intestinal conditions, including constipation, since it absorbs large quantities of water to form colloidal solutions, and otherwise has properties similar to cellulose.



The oxidation of cellulose by  $\text{NO}_2$  under appropriate conditions yields an oxidized cellulose which is soluble in slightly alkaline solutions. Probably primary hydroxyl groups are attacked, yielding oxidation products containing combined uronic acid units. These cellulose products retain their fibrous structure and will undoubtedly find uses in medicine. Already some applications have been suggested. Pledgets of this cotton containing thrombin, the blood-clotting principle, have been applied to wounds and left there. The mass is more adherent than a clot alone would be and is eventually dissolved by the faintly alkaline body fluids and is then absorbed. Absorbable paper and gauze have also been tested in animal experiments. When left in contact with brain, muscle, joints, or other locations they are also absorbed after serving their purpose of supporting or protecting injured surfaces where a smooth membrane is desired in the final healing.

**INULIN.**—Inulin is a fructosan. It is a starchlike polysaccharide which occurs in the tubers of the dahlia, in the roots of the Jerusalem artichoke, the dandelion, and chickory, and in the bulbs of onion and garlic. It is a white tasteless powder, levorotatory, and gives no color with iodine. Acids hydrolyze it to D-fructose, as does inulase, the enzyme which accompanies it in plants. For a while, the use of inulin was recommended in the dietary of individuals with diabetes on the assumption that it would be digested to fructose, which was further assumed to be more easily utilized by persons suffering from diabetes. It is not certain whether it is digestible although it is possible that fructose is handled better than glucose in diabetes. Fructosans are being discovered continually in various plant materials. They are found in large amounts in grasses and in smaller amounts in cereals.

**HEMICELLULOSES.**—The pentosans, galactans, mannans, and similar polysaccharides have long been termed hemicelluloses. They are probably of smaller molecular size than the celluloses, whence the name. The pentosans have been considered before. Agar-agar, a product derived from certain seaweeds, contains a large amount of galactan. Agar is used in the preparation of cultural media in bacteriology. It is not digested in the human alimentary tract and therefore it is of value in treating certain forms of constipation, acting as a soft, nonirritating type of roughage. Mannans are also quite indigestible. Some mannan is found in the carob bean, or St.-John's-bread, and also in the ivory nut.

**MIXED POLYSACCHARIDES.**—Mixed polysaccharides are designated "hemicelluloses" by some authorities, but it seems wiser to reserve that term for the group just described. The molecules of a mixed polysaccharide are composed of both pentose and hexose units together with an uronic acid (see page 73) such as glycuronic acid. The more complex ones are found in gums, mucilages, wood, and other plant tissues frequently associated with other polysaccharides. Most of the plant materials are not made up of any one pure carbohydrate. For example, gum arabic contains a nucleus relatively resistant to acid hydrolysis. This is composed of galactose and glucuronic acid units. With them are combined, more loosely, arabinose and methyl pentose, which can be easily split off by mild treatment with acids. Slippery elm mucilage and flaxseed



mucilage both appear to contain rhamnose and galacturonic acid, but other carbohydrates are probably admixed.

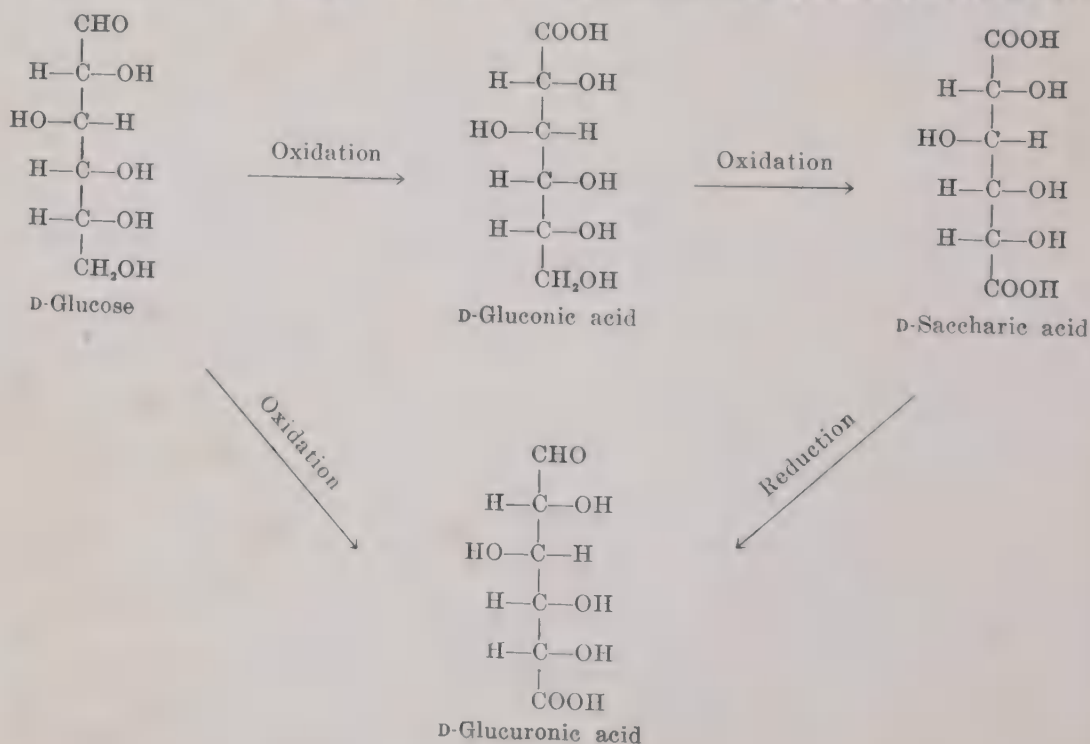
The mixed polysaccharides constitute the major part of the indigestible residue of foods and therefore also become a part of the roughage. Those polysaccharides which are not digested and are not easily hydrolyzed by acid or alkali are designated "fiber" in reports of food analyses. The higher mixed polysaccharides are, of course, in this group, and also those hemicelluloses which are not soluble in water.

Pectins, or, more properly, "pectic substances," are the materials responsible for the gelling properties of fruits. A suitable concentration of acid, sucrose, and pectin is necessary to produce a jelly. Whether these carbohydrates may be classed as mixed carbohydrates is very doubtful now. They appear to yield, on hydrolysis, arabinose, galactose, acetic acid, methyl alcohol, and galacturonic acid, and various hypotheses have been advanced to account for all these constituents. Recent work indicates, however, that the arabinose and galactose are derived from arabans and galactans associated with the pectin. The term "pectic substance" is now used as a group designation for these complex carbohydrate derivatives. They contain a large proportion of methylated galacturonic acid molecules, which are believed to exist in long chains.

### Carbohydrate Derivatives

On hydrolysis of certain more complex substances, amino sugars are obtained. In these, the amino group replaces an hydroxyl group. Thus we obtain 2-amino glucose as one of the products of hydrolysis of a glycoprotein, and 2-amino galactose from chondroitic acid, a constituent of cartilage.

Oxidation of the monosaccharides yields, among others, three important types of acid. Using D-glucose as an example, these may be shown as follows:



Saccharic acid is probably not formed in the body but is produced *in vitro*. It is the analog of mucic acid, formed from galactose, but it is soluble, whereas mucic acid is not very soluble. Gluconic and glucuronic acid are produced metabolically. Glucuronic acid sometimes is found in urine, usually in conjugated forms. The glucuronides do not reduce. However, glucuronic acid in the free form has reducing properties and is dextrorotatory, but cannot be fermented by ordinary yeast. It combines with and "detoxicates" various toxic compounds which may be formed in the body or may be absorbed from the intestinal tract. (See page 648.) With certain sex hormones it forms compounds which are much more soluble in the aqueous body fluids than the uncombined hormones. Acids of this type are called "uronic acids." Complexes of the uronic acids with glucosamine, sulfuric acid, or acetyl or other groups are found in the skin and other parts of the body as well as in the capsules of various cocci. These "mucopolysaccharides" may occur free or in combination with proteins. Chondroitin sulfuric acid, heparin, and hyaluronic acid are important examples which will be discussed in subsequent sections. Condensed uronic acids, polyuronides, are formed by bacilli and by plants. In this form they are constituents of vegetable foods.

As mentioned previously (page 20), compounds containing the uncommon isotope of one of its constituent elements are very useful in biochemical studies. The synthetic preparation of carbohydrates containing radioactive carbon is, however, extremely difficult. A novel method of producing starch, sucrose, glucose, or fructose so labeled has been described. (E. W. Putnam.) Green leaves are placed in water in the dark to use up reserve carbohydrates. They are then exposed to light in an atmosphere containing carbon dioxide tagged with radioactive carbon. Photosynthesis results in the formation of one or more carbohydrates containing labeled carbon in the molecules. Different plant leaves produce different radioactive carbohydrates.

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## Chapter 4

### LIPIDS

The lipids constitute a very important group of organic substances in plant and animal tissues, a group which is difficult to characterize clearly. Its members have certain solubilities and properties in common but are rather diverse in their chemical constitution. Lipids include the fats and other compounds which resemble them in physical properties. The terminology in this field is in a rather confusing state and various names other than lipid, such as lipoid and lipin, have been suggested for this group of compounds. Lipid seems to be the one preferred by most biochemists and was suggested by Bloor. It was also recommended by the International Congress of Pure and Applied Chemistry. According to Bloor, lipids are compounds having the following characteristics: a. Insolubility in water and solubility in one or more organic solvents, such as ether, chloroform, benzene, acetone—the so-called “fat solvents.” b. Some relationship to the fatty acids as esters, either actual or potential. c. Possibility of utilization by living organisms.

Bloor's classification is quite generally adopted in this country and is as follows:

#### I. Simple lipids—Esters of fatty acids with various alcohols

1. Neutral fats: esters of fatty acids with glycerol
2. Waxes: esters of fatty acids with monatomic alcohols higher than glycerol
  - (a) True waxes: products of both animal and vegetable origin in which the esters are composed of palmitic, stearic, oleic, or other higher fatty acid esters of cetyl alcohol ( $\text{CH}_3[\text{CH}_2]_{14}\text{CH}_2\text{OH}$ ) or other higher straight chain alcohols.
  - (b) Cholesterol esters: esters of fatty acids with cholesterol
  - (c) Vitamin A esters: palmitic or stearic acid esters of vitamin A
  - (d) Vitamin D esters

#### II. Compound lipids—Esters of fatty acids with alcohols plus other radicals

1. Phospholipids: Lipids containing phosphoric acid and a nitrogenous base
2. Glycolipids or cerebrosides: Lipids containing a carbohydrate and also nitrogen but no phosphorus and no glycerol
3. Sulfolipids: Lipids characterized by possessing sulfate groups

#### III. Derived lipids—Derivatives obtained by hydrolysis of those given in Groups I and II which still possess the general physical characteristics of lipids

1. Saturated and unsaturated fatty acids
2. Monoglycerides and diglycerides



## 3. Alcohols

- (a) Straight chain alcohols: water-insoluble alcohols of higher molecular weight obtained on hydrolysis of waxes
- (b) Sterols
- (c) Alcohols containing the  $\beta$ -ionone ring: these include vitamin A and certain carotenols

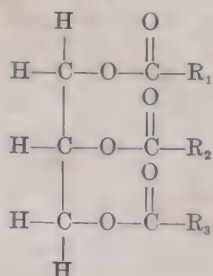
## 4. Hydrocarbons

- (a) Aliphatic hydrocarbons: these include iso-octadecane found in liver fat and certain hydrocarbons found in beeswax and plant waxes
- (b) Carotenoids
- (c) Squalene: a hydrocarbon found in shark liver oil and in human sebum
- (d) Vitamins D, E, and K

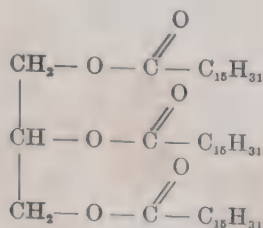
**FATS**

As stated in the classification, fats are all esters of the trihydric alcohol, glycerol, and certain, but not all, organic acids.

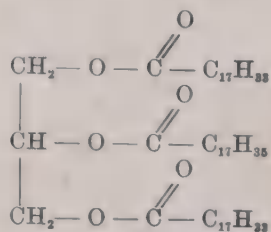
The type formula for a fat is:



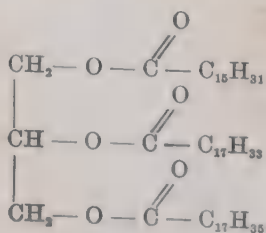
$\text{R}_1$ ,  $\text{R}_2$ , and  $\text{R}_3$  represent fatty acid chains which may or may not all be the same. Since all three of the glycerol alcohol radicals are esterified, fats are termed "triglycerides." Some typical fats are:



Tripalmitin



Stearo-diolein



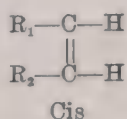
Palmito-oleostearin

It is questionable whether natural fats consist of mixtures of pure glycerides like tripalmitin. It is more probable that they are really mixtures of mixed glycerides as represented by stearo-diolein and palmito-oleostearin, in which the fatty acid residues are all different or there are at least two different ones to the molecule. A number of mixed glycerides have been isolated from natural fats. The most common fatty acids occurring in the fats are:

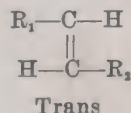
Saturated	{	Butyric	$C_4H_7COOH$
		Caproic	$C_6H_{11}COOH$
		Palmitic	$C_{16}H_{31}COOH$
		Stearic	$C_{17}H_{35}COOH$
Unsaturated	{	Oleic	$C_{17}H_{33}COOH$
		Linoleic	$C_{17}H_{31}COOH$
		Linolenic	$C_{17}H_{29}COOH$

Many other fatty acids of both series are found in the naturally occurring fats. Capric, lauric, myristic, and arachidic acids may be included in the group of saturated fatty acids and elupanodonic and arachidonic acids in the group of unsaturated fatty acids. Two hydroxy acids, ricinoleic and dihydroxystearic acid, are constituents of fats in castor oil. Two cyclic acids, hydnocarpic acid and chaulmoogric acid, are of interest because chaulmoogra oil, in which they are combined, is used in the treatment of leprosy.

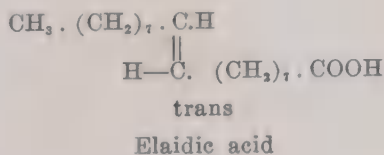
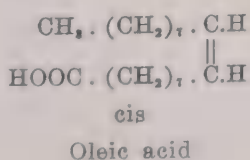
Isomerism of fatty acids may be of several types. The saturated chains may be straight chains or branched. Thus, there is normal butyric acid,  $CH_3.CH_2.CH_2.COOH$ , and its isomer, isobutyric acid,  $\begin{matrix} CH_3 \\ | \\ CH_3 \end{matrix} > CH.COOH$ . In unsaturated fatty acids, isomerism may be due to the position of the double bond in the chain. Still another type of isomerism depends upon the spacial arrangement. The double bond limits the free rotation of the carbon atoms at this linkage and therefore two forms are possible. If  $R_1$  and  $R_2$  represent the two ends of the molecular structure, we have two forms, termed *cis* and *trans* forms:



and



Oleic acid has such an isomer which is useful in physiological experiments, because the body apparently does not distinguish between them. However, the isomer, elaidic acid, has characteristic differences which permit its separate isolation and analysis. Therefore, if elaidic acid is administered to an animal its travels can be followed and one can infer how oleic acid is handled under the same conditions.



### Physical Properties of Fats

The hardness, or consistency, of fats is related to their melting points, which are not sharp, since the natural fats are mixtures rather than pure substances. The solidification points are considerably lower than the melting points. Glycerides of the lower fatty acids melt at lower temperatures than those of the higher fatty acids, and the unsaturated fatty acid glycerides still lower. Many of these, triolein, for example, are liquid at room temperatures and are commonly called "oils." The term "oil" is rather confusing because it is often used for substances having no relation to the lipids, such as mineral oil, which is a mixture of hydrocarbons, and oil of vitriol, which is concentrated sulfuric acid. It should, therefore, be understood that the word "oil" indicates the physical state of a substance, not its chemical nature. The hardness of common fats depends largely on the relative amounts present of fats containing long-chain saturated fatty acids, like palmitic and stearic acids, and those containing unsaturated fatty acids like oleic and linoleic acids. The former are solid and the latter liquid at room temperature. There are larger proportions of the soft fats in cold-blooded animals than in warm-blooded animals. This facilitates motility at low temperatures.

The specific gravity of all fats is less than 1.0. Consequently all fats float on water. They are not soluble in water, or at least not to any appreciable extent.

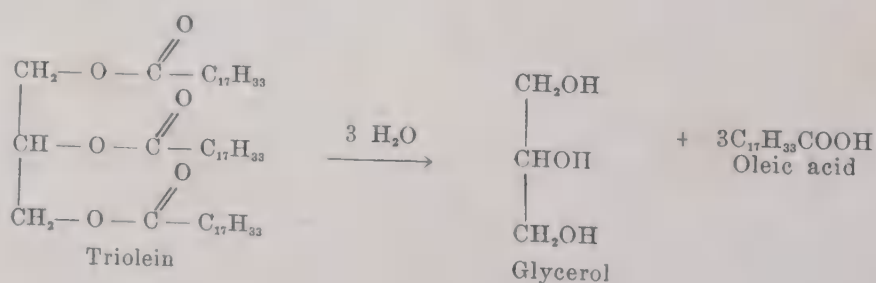
Emulsions of fats may be made by shaking vigorously in water, but, of course, emulsifying agents such as gums, soaps, and proteins produce more stable emulsions. The emulsification of fats in the intestinal canal is a prerequisite for digestion and absorption. All fats are soluble in ether, chloroform, and benzene, as well as in hot ethyl alcohol and hot acetone.

The flavor of food fats is attributed to foreign substances absorbed by the fat either from its natural environment or from materials produced during processing. For example, in the manufacture of butter, the bacterial flora are carefully controlled in order to impart the distinctive flavor to the food.

The color of human body fat, as well as that of human milk fat, is derived from carotene and xanthophyll present in the diet. The amount of these plant pigments is very small, only 5 or 6 mg. being present in a kilogram of fat.

### Hydrolysis of Fats

The fats may be hydrolyzed by superheated steam, by alkalies, or by the specific fat-splitting enzymes, the lipases. They yield glycerol and the constituent fatty acids:



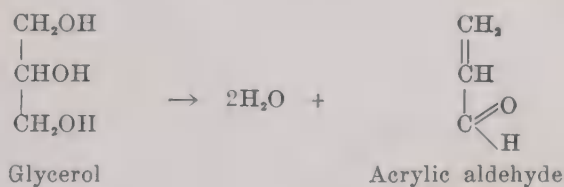
If alkali is the agent used, the alkali salts, or soaps, are formed.



In this type of reaction, the hydrolysis is called a saponification. Soaps can, of course, also be formed by causing alkali to react with the fatty acid. Both products of saponification are of interest from several aspects.

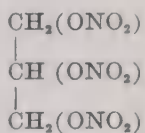
### Glycerol

Glycerol, commonly called "glycerin," is the simplest trihydric alcohol. It is a colorless, oily fluid with a sweetish taste. Besides being a by-product in soap manufacture, it is also obtainable in the fermentation of glucose by changing conditions in such a way as to decrease the formation of  $\text{CO}_2$  and alcohol. It is miscible with water and alcohol in all proportions but is almost insoluble in ether. With dehydrating agents, acrylic aldehyde, or "acrolein," is formed.



Acrolein has a very acrid odor and therefore is easily detected. Any compound containing glycerol will give an acrolein test.

Glycerol finds many uses in industry as a result of its solubility, its solvent action, and its hygroscopic nature. Many pharmaceutical and cosmetic preparations have glycerol in their formulas. When treated with nitric acid, it forms glyceryl trinitrate, or "nitroglycerin"



This is an important explosive either alone or as a constituent of dynamite and smokeless powders. In medicine, nitroglycerin is a vasodilator, of great value in diseases of the circulatory system.

Physiologically, glycerol, a product of fat digestion, has a definite nutritive value. It has about the same caloric value as the sugars and probably follows a similar course when it is utilized by the cells of the body.

### Soaps

Soaps are salts of the nonvolatile acids whose esters form the fats. However, the common soaps are those of sodium and potassium. Sodium soaps are the ordinary hard soaps, while potassium soaps are soft. When potash was



cheaper than soda, soft soap was a common household article, but there is no longer any point in using the less convenient and now more expensive potassium soap.

The floating soaps are made light by beating air bubbles into the hot melted soap and then chilling it and trapping the air. Most household soap has sodium carbonate or sodium silicate added to overcome the hardness of water. Scouring soap has an abrasive added, and laundry soap may have naphtha or other special ingredients. Most toilet and household soaps have perfume added, not only for the pleasing aroma, but also to prevent the soaps from becoming rancid. Transparent soap acquires this property by the incorporation of sugar. Shaving soaps are in part potassium soaps of coconut and palm oils. Castile soap is a sodium olive oil soap and green soap is sodium and potassium linseed oil soaps mixed. Many household soaps contain a certain amount of soap prepared from rosin mixed with the ordinary type derived from fats.

In general, soaps have a slightly alkaline reaction because of hydrolysis.



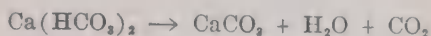
In order to prevent irritation of the skin as a result of this alkalinity, toilet soaps sometimes are modified by adding an excess of fatty acids or a larger proportion of sodium oleate. The latter is not as readily hydrolyzed as most of the other sodium soaps.

The heavy metals produce insoluble soaps which are of relatively little importance. Zinc stearate is an exception. This is a white powder having a greasy feeling. It is soft, water-repellent, and mildly antiseptic and astringent and is used as a dusting powder, particularly for babies.

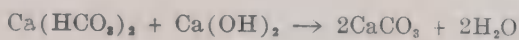
The cleansing action of soaps is probably due to the fact that they are very effective emulsifying agents. Most dirt is held to surfaces by greasy substances. By emulsifying and washing away the grease the dirt is carried away. Soapy solutions also can wet and penetrate into oily textures because of their low surface tension. This is an additional aid in the cleansing action.

**Insoluble Soaps and Hard Water.**—Ordinary sodium soaps are not very soluble, although we usually consider them so. They are easily precipitated by strong salt solutions which is one reason why the common soaps are useless in sea water. The calcium and magnesium soaps are even less soluble, and if sodium soap is added to water containing these ions, the soap is immediately precipitated as the insoluble calcium or magnesium soaps, which, of course, do not lather or cleanse. Water containing either or both calcium and magnesium ions is called hard water. Hard water is not harmful for drinking purposes. If it contains more than ordinary amounts of salts, it may have a slightly salty flavor which some people like and others do not. The main objection to it arises from its precipitation of soap. This continues until all the calcium and magnesium ions are combined. Additional soap will then permit

of lathering, but this means that more soap will be required. Second, the precipitated calcium and magnesium soaps cling to washed materials and cause them to be harsh and irritating to a sensitive skin. Hardness may be "temporary" or "permanent." Temporary hardness is due to the bicarbonates of calcium or magnesium and is so-called because the water may be softened by boiling. The bicarbonate is decomposed and the carbonate precipitated.



Permanent hardness is caused by the presence of such salts as are not changed by boiling; e.g., the chlorides. Temporary hardness is more conveniently abolished by the use of slaked lime.



There are several salts which will soften permanently hard water, among them sodium carbonate, borax, and trisodium phosphate.



A combination of lime and sodium carbonate is utilized industrially (the Porter-Clark process). The resulting insoluble calcium and magnesium compounds are filtered off. Another method is more adaptable to homes, laundries, and hospitals and is gaining in popularity. It is the permutit process. Permutit is an artificial zeolite—sodium aluminum silicate,  $\text{Na}_2\text{Al}_2\text{Si}_2\text{O}_8$ —which we may represent as  $\text{Na}_2\text{Zeo}$ . In contact with hard water an exchange of the Ca or Mg for the Na occurs, and the water is thereby softened.



This is accomplished by permitting the hard water to filter through a column of the zeolite. Eventually the zeolite will become depleted of all its sodium. It is then regenerated by allowing a sodium chloride solution to filter through it.



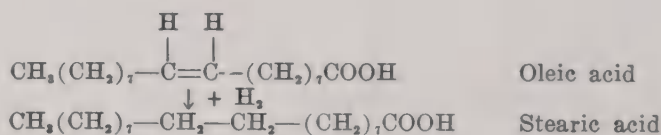
After the initial installation the only cost is that of the sodium chloride needed for regeneration. This is very little in comparison with the cost of soap saved by the water-softening operation. In toilet soaps coconut oil is added to the other fats to increase the lathering property, and for marine and hard-water soaps both palm and coconut oils are used in greater proportion. These oils are rich in laurin, caprin, caprylin, and caproin, and the soaps derived from these fats are not as easily salted out as those derived from the common fats, and their calcium and magnesium soaps are much more soluble.

**The New Detergents.**—The use of the newer "nonsoap" detergents has found rather wide application in the home, in industry, and in medicine. Most of the detergents fall into three groups: (1) The *anionic* are exemplified by certain alkyl sulfates, such as the sodium

salt of lauryl sulfuric acid, sulfated esters, such as the sulfated lauryl monoglyceride, sulfated amines, and various sulfonates. (2) The *cationic* group contains quaternary ammonium salts containing a long aliphatic chain. (3) Among the *nonionic* detergents are esters and ethers, such as "Tween 40," the palmitic acid ester of a sorbitan polyethylene derivative. These are all good wetting agents and emulsifiers and therefore good cleansers. Since they are not soaps, they are independent of the hardness of water and are being used in increasing volume in the home. Furthermore, soaps sometimes cause dermatitis or may be irritating to diseased skin, and a nonsoap detergent may be desirable. Some of these detergents have also been shown to be nontoxic when taken by mouth and have been used as agents to bring into solution or emulsion difficultly soluble foods or remedial substances.

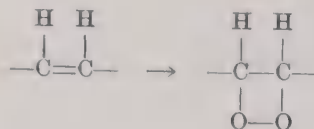
### Unsaturation

Fatty acids which contain one or more double bonds in their chains are said to be unsaturated. In the oleic acid series there is only one such unsaturated linkage. Oleic acid is the most widely distributed acid of this or any other fatty acid series. Its double bond,  $-\text{CH}=\text{CH}-$ , occurs exactly in the middle of the chain. By adding hydrogen, the unsaturated acids are converted to saturated.



Hydrogenation changes the liquid oleic acid, which solidifies at about  $3^\circ \text{C}$ ., to solid stearic acid, which melts at  $60$  to  $65^\circ \text{C}$ . In this way soft fats containing large proportions of triolein are converted into hard fats. The hydrogenation is catalysed by finely divided nickel. This transformation of soft fats of vegetable or animal origin into more savory cooking fats and margarines is a large industry in this country.

Unsaturated fatty acids are also oxidizable at the point of unsaturation. They form peroxide derivatives



and also to some extent other derivatives, such as aldehydes, ketones, and acids, having smaller chains. The "drying oils," used in paints, owe their peculiar property to the fact that they contain highly unsaturated oils which on atmospheric oxidation are converted to hard films. Linseed oil is a familiar example.

### Rancidity

Most fats on exposure to air develop an unpleasant odor and flavor. This development of rancidity results from the hydrolysis of the fat, probably to only a slight extent. This leads to the liberation of volatile fatty acids having



more or less unpleasant odors. Simultaneous oxidation of the unsaturated acid occurs with the formation of the peculiar oxidation products mentioned. Light, heat, moisture, and bacterial action are all factors which tend to bring about rancidity. Besides their disagreeable properties, the rancid fats and oils may have distinctly unphysiological effects by oxidizing a number of essential dietary substances (see Chapter 17). The rate of production of rancidity varies with the individual fat, as well as being influenced by bacterial growth, etc. There are present in the nonsaponifiable fraction substances which inhibit the auto-oxidation of the fats. They are called "antioxidants" and occur in different concentrations in the various natural fats. This explains why some fats keep better than others. Compounds possessing this property include certain phenols, naphthols, and quinones (Mattill). The most common natural antioxidant is, perhaps, vitamin E. It is often added to foods and other materials to prevent the production of rancidity.

### Identification of Fats and Oils

It is frequently necessary to identify a pure fat or to determine the proportions of different types of fat in a mixture. Besides the melting and congealing points, several other values may be ascertained. These depend upon certain chemical, physical, and structural characteristics of the fatty acid fraction. The more important are the iodine number, the saponification number, the Reichert-Meissl number, and the acetyl number.\*

**Iodine Number.**—The unsaturated fatty acids take up iodine and other halogens at the point of unsaturation, yielding saturated halogen derivatives. Consequently, the degree of unsaturation of fats may be determined by ascertaining how much iodine a given quantity will absorb. The result is called the "iodine number." This is defined as the number of grams of iodine that is absorbed by 100 grams of fat. The determination of the iodine number is very useful to the chemist in determining the quality of an oil or its freedom from adulteration. For example, the iodine number of cottonseed oil varies from 103 to 111, that of olive oil from 79 to 88, and that of linseed oil from 175 to 202. A commercial lot of olive oil which has an iodine number somewhat higher than 88 might have been adulterated with cottonseed oil. Again, a shipment of linseed oil with an iodine number lower than 175 might also have been adulterated with the same oil.

**Saponification Number.**—Since each carboxyl of a fatty acid reacts with one molecule of NaOH or KOH in a saponification, it is evident that the amount of alkali needed to saponify a given quantity of fat will depend upon the number of carboxyls present. Fats containing short-chain acids will have more carboxyls per gram than long-chain acids and will take up more alkali. The "saponification number" therefore becomes another criterion of value, giving a

\*The procedures for these as well as many other quantitative methods in food analysis will be found in *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, ed. 6, Washington, 1945, Association of Official Agricultural Chemists.



clue as to the average size of the fatty acid chain in the fat under investigation. It is defined as the number of milligrams of KOH necessary to neutralize the fatty acids in 1 gram of fat. Butter, containing a larger proportion of short-chain fatty acids, such as butyric and caproic acid, than other common fats, has the relatively high saponification number of 220-230. Oleomargarine, with more long-chain fatty acids in its composition, has a saponification number of 195 or less.

**Reichert-Meissl Number.**—This measures the amount of volatile soluble fatty acids. By saponification of the fat, acidification, and steam distillation, the volatile soluble acids may be separated and determined quantitatively. The Reichert-Meissl number is the number of cubic centimeters of 0.1 N alkali required to neutralize the soluble fatty acids distilled from 5 grams of fat. Butterfat is the only common fat with a high Reichert-Meissl number and this determination, therefore, is of interest in that it aids the food chemist in detecting butter substitutes in food products.

**Acetyl Number.**—Some of the fatty acid residues in fats contain hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride. The acetylated fat is then saponified with KOH. The fatty acids are liberated by  $H_2SO_4$  and the acetic acid is separated from the insoluble fatty acids and titrated with standard alkali. The acetyl number, which is thus a measure of the number of hydroxyl groups present, is the number of milligrams of KOH needed to neutralize the acetic acid of 1 gram of acetylated fat. Examples of the values for certain oils are given below. The applications to adulteration are evident.

ACETYL NUMBER		ACETYL NUMBER	
Castor oil	146-150	Cottonseed oil	21-25
Cod-liver oil	1.1	Olive oil	10.5
		Peanut oil	3.5

### Essential Fatty Acids

As will be seen later, the fats have a very high value as sources of energy to the body. Besides this, certain fatty acids appear to have specific nutritional importance. Burr and Burr have shown that skin lesions occur in rats on a fat-deficient diet and that these may be cured by the addition of linolenic, linoleic, and arachidonic acids, or of fats containing these unsaturated acids. Although animals are capable of desaturating fatty acids, and thus producing unsaturated acids, they do not seem to be able to form these particular ones. Therefore, these fatty acids may be called essential. Steenbock and his co-workers have reported that these same fatty acids have curative properties for the skin affections caused by lack of vitamin  $B_6$ , which can, of course, also be cured by administration of this vitamin. This does not mean that the unsaturated fatty acids are vitamins, but that they undoubtedly play some important role, as yet undetermined, in metabolism.

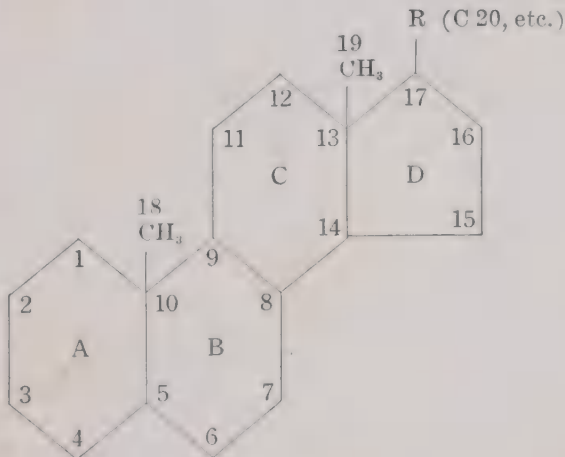
## WAXES

Waxes are esters of fatty acids with certain alcohols—not glycerol and not the sterols. They are insoluble in water but are soluble in the fat solvents. They are not as easily hydrolysed as the fats and are not digested by the fat-splitting enzymes. Therefore, they are of no value from a nutritional standpoint. Examples are beeswax, spermaceti, Chinese wax, and carnauba wax.

Beeswax is secreted by the honeybee to form the comb. It is a mixture of waxes, the chief ingredient being myricyl palmitate. Spermaceti is likewise a mixture. It is found in the skull of certain whales and dolphins. It is chiefly cetyl palmitate and was formerly used in the manufacture of candles. Chinese wax and carnauba wax are derived from the cuticle of leaves. These and other vegetable waxes are of value from an industrial standpoint as ingredients of shoe polish, varnishes, candles, etc.

## STEROLS

Sterols are complex monohydroxy alcohols found in both plant and animal tissues. They belong to the group of compounds known as cyclopentanoperhydrophenanthrenes, which have recently been designated “steroids.” These have a four-ring structure, which is shown below with the rings lettered and the carbon positions numbered. R indicates an aliphatic side chain.



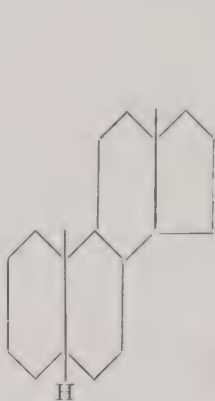
Steroid Nucleus

Those carbons numbered above 17 are not present in every steroid compound. The steroids differ from each other in the arrangement of double bonds in the rings and in the presence of oxygen or of hydroxyl or other groups, and, in certain cases, there may even be a break in one of the rings. This numbering system is frequently referred to in biochemical and clinical literature. Among the steroids are included the sterols, the bile acids, the sex hormones, the adrenal cortical hormones, the cardiac aglycones, and the D vitamins.

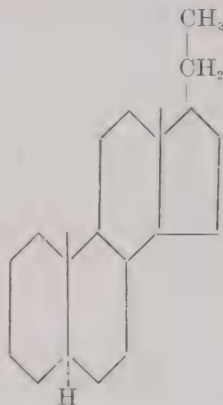
Many of the steroids are known by common or “trivial” names which do not indicate their structure but which are retained because of long usage;

e.g., androsterone, progesterone, estrone. They, as well as the other steroids, can be given more exact designations which will describe their formulas rather clearly. The system of nomenclature now generally accepted is as follows (see Mason; Fieser and Fieser; Dorfman and Ungar):

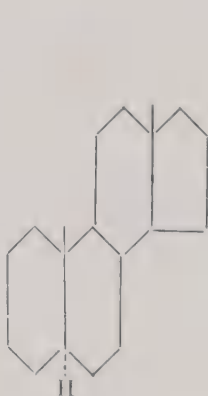
There are a number of hydrocarbons which are the parent substances of different series of compounds. The ending of their names is *ane* and the most important ones are:



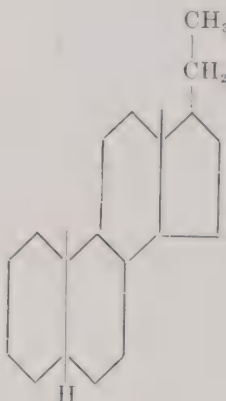
Etiocholane



Allopregnane



Androstane



Pregnane



Estrane

The vertical bonds at the 10 and 13 positions indicate methyl groups with C18 and C19, respectively.

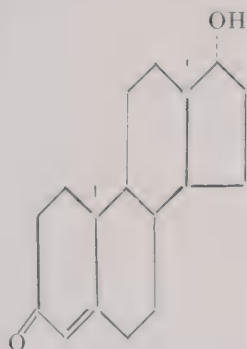
An unsaturated bond changes the name of the hydrocarbon to *ene*; two such bonds make it *diene*, etc. The exact position of the double bond is shown by the Greek letter  $\Delta$  with a superscript numeral to indicate where the double bond starts; e.g.,  $\Delta^5$ -androsterone. The number of the carbon at the end of the double bond is also given if it is not the next higher number; e.g.,  $\Delta^{7,14}$ -androstadiene indicates that the two double bonds go from C7 to C8 and from C14 to C15, while  $\Delta^{7,9:11}$ -androstadiene means that the second double bond in this case goes from C9 to C11 (not from C9 to C10).

If an oxygen atom is introduced in place of a hydrogen, the suffix is changed to *one*, or the syllable *one* is added, with a number to indicate its po-

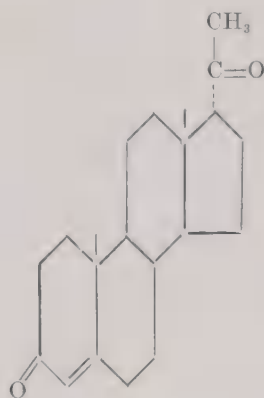
sition. Similarly *ol* is used for a hydroxyl; e.g.,  $\Delta^{1,3,5}$ -estratriene-3-ol-17-one indicates that the compound has the estrane configuration but with three double bonds from C1 to C2, C3 to C4, and C5 to C6; it also has a hydroxyl at C3 and an oxygen at C17.

It is obvious that there are many asymmetric carbons in the steroid molecule and that in some cases it might be necessary to show precisely how a given substituent is oriented. Configurations relative to the molecule as a whole are designated  $\beta$  if the orientation of the hydrogen or group corresponds to that of the two methyl groups C18 and C19 which are presumed to be above the plane of the page. A full line bond is used for such an orientation. If the orientation is opposite the C18 and C19 groups, i.e., below the plane of the page, it is called  $\alpha$ , and a dotted line bond is used. The terms *trans* and *cis* are also applied to  $\alpha$  and  $\beta$ , respectively.

The principles just presented will be understood if the following structures are compared with the names beneath them.



Testosterone

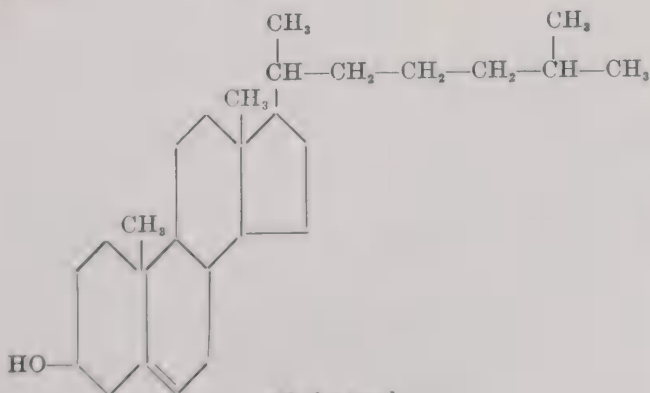
 $\Delta^4$ -Androstene-3-one-17( $\alpha$ )ol

Progesterone

 $\Delta^4$ -Pregnene-3,20-dione

### Cholesterol

Cholesterol is perhaps the most important sterol; it is very widely distributed and has been known and studied for many years. Its structural formula is:



Cholesterol



Since there are eight asymmetric carbons, there are, theoretically, 256 possible stereoisomers. Cholesterol is probably a constituent of all animal cells; the corpus luteum and the adrenal cortex are particularly rich in this lipid. It is present in blood and bile and is usually a major constituent of gallstones from which it was first isolated. The name cholesterol is from the Greek words meaning "solid bile." For study it may be readily obtained from gallstones or from nervous tissue, where it is also found in high concentration. It is soluble in many "fat solvents," such as ether, chloroform, benzene, and hot alcohol, and easily crystallizes from such solutions in colorless rhombic plates with one or more characteristic notches in the corners (see Fig. 9). Since it has an unsaturated bond, it will take up two halogen atoms. It is not saponifiable.

Cholesterol gives a number of color reactions. These enable one to test for it both qualitatively and quantitatively. A beautiful series of colors is obtained by the Salkowski reaction. The chloroform solution of cholesterol is stratified over concentrated sulfuric acid. The acid assumes a yellowish color with a green fluorescence, while the chloroform layer becomes first bluish red, then gradually violet-red. If the chloroform solution is poured into a porcelain evaporating dish, it changes to violet to green to yellow. If to a chloroform solution of cholesterol there are added acetic anhydride and concentrated sulfuric acid (under as nearly anhydrous conditions as possible) a blue to violet color

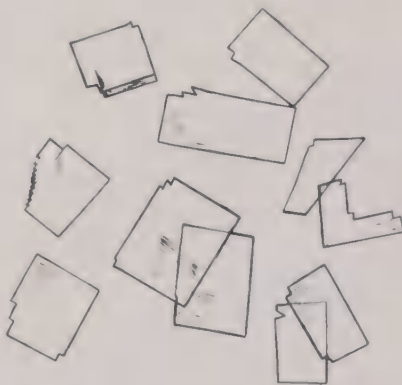


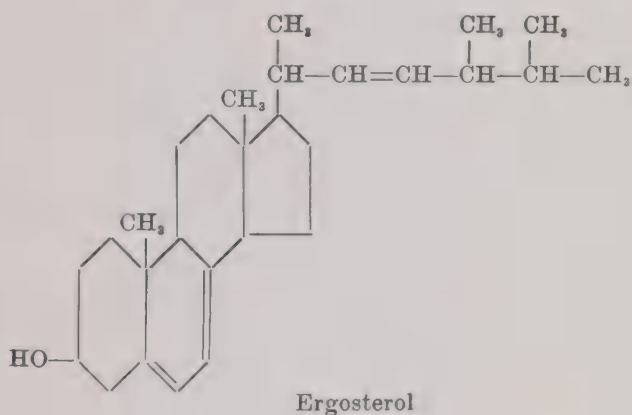
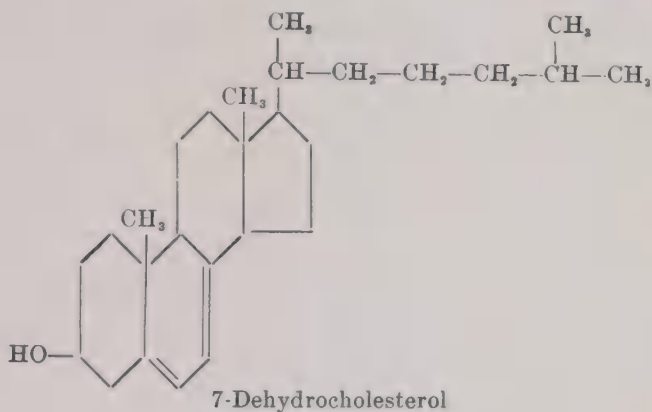
Fig. 9.—Cholesterol crystals.

appears, which changes to emerald green. Under carefully controlled conditions the green color produced is proportional to the amount of cholesterol present. Consequently this reaction, the Liebermann-Burchard reaction, has become the basis for the quantitative estimation of cholesterol in blood and other biological materials. In clinical work it is sometimes necessary to determine free cholesterol and cholesterol esters separately. In order to accomplish this, advantage is taken of the fact that free cholesterol unites with digitonin to form cholesterol digitonide. This is insoluble in petroleum ether, in which the cholesterol esters are freely soluble (Bloor and Knudson).

#### Other Important Sterols

There is present beneath the skin an important sterol, 7-dehydrocholesterol. This differs from cholesterol only in having a second double bond, namely,

between the C7 and C8, and, therefore, only one H at C7 and none at C8. It is found in other tissues as well as the skin, probably along with cholesterol, but its special interest lies in the fact that when the skin is irradiated with ultra-violet light, this sterol is converted to one of the D vitamins. This explains the value of sunshine in preventing rickets.



In this connection mention should be made of ergosterol. This sterol has the same nucleus as 7-dehydrocholesterol but differs slightly in its side chain. It may also be converted to a vitamin D by irradiation with ultraviolet. Each of these sterols, therefore, is called a "pro-vitamin D." Ergosterol was first isolated from ergot, a fungus of rye, and later from yeast and certain mushrooms. Stigmasterol and sitosterol are among the other sterols occurring in the higher plants, but there is at present no evidence that they have any nutritional value for man. On the other hand, the sterols of animal origin, notably cholesterol, are probably absorbed from the intestinal canal and utilized. However, all animals, herbivorous as well as carnivorous, can synthesize cholesterol from other dietary factors. This must occur to a very considerable degree, because whether or not cholesterol is in the diet, it is continually being excreted by way of the bile. No doubt some of this is reabsorbed, but some continues down the intestinal tract and is largely converted to coprosterol. Coprosterol is formed by the hydrogenation of the double bond of cholesterol. This is probably brought about by bacterial

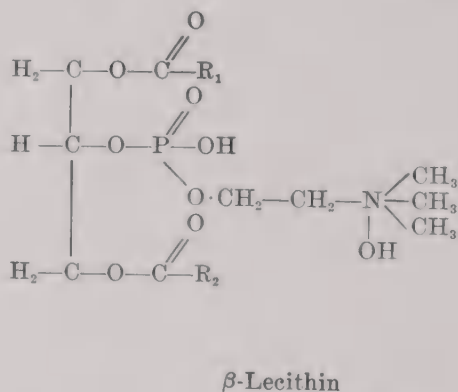
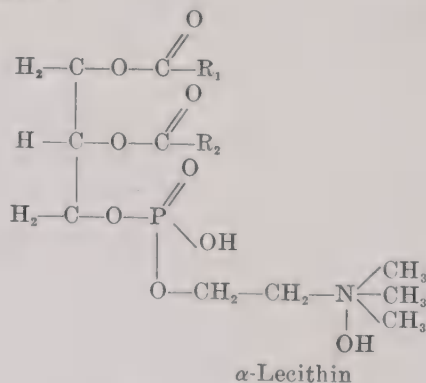
action. Thus, feces contains coprosterol, cholesterol, and the plant sterols. Coprosterol is soluble in chloroform and gives similar, but not identical, color tests to those given by cholesterol. Of course, it does not take up halogens.

## PHOSPHOLIPIDS

The phospholipids, like the sterols, are probably present in all cells, plant as well as animal. They are also known as phosphorized fats, phospholipins, and phosphatides. Most of them are composed of fatty acids, a nitrogenous base, phosphoric acid, and glycerol, inositol, or sphingosine. Three types are of interest; i.e., lecithins, cephalins, and sphingomyelins.

### Lecithins

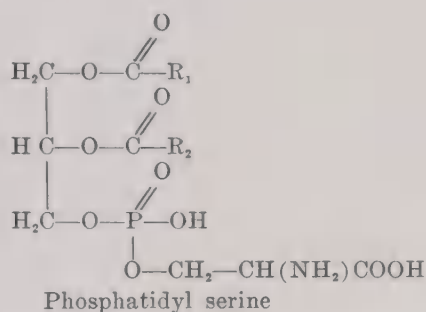
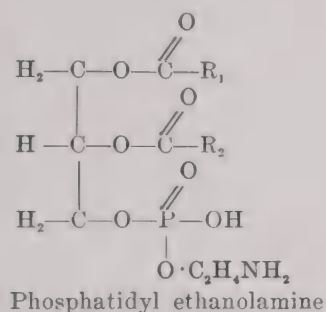
On hydrolysis, a molecule of lecithin will yield two molecules of fatty acid and one molecule each of glycerol, phosphoric acid, and choline. Choline is ethanol trimethylammonium hydroxide, or trimethyl hydroxyethylammonium hydroxide. The following formulas show how there may be two types of lecithins, depending upon the point of linkage of the phosphoric acid to the glycerol:



The  $\text{R}_1$  and  $\text{R}_2$  represent such fatty acid chains as oleic, stearic, etc. There are accordingly many different lecithins. The lecithins are soluble in many fat solvents including ether, chloroform, benzene, and hot alcohol. *They are not soluble in acetone*; this property is used in separating them (and other phospholipids) from cholesterol and fats. Although insoluble in water, they readily emulsify in it and have a great affinity for it. When first prepared, they are white waxy solids but are quickly oxidized and become very dark in color. The lecithins may be saponified by alkalis, which completely disrupt the molecule, yielding glycerol, soaps, choline, and phosphate. They may also be hydrolyzed by lecithinases, specific enzymes which attack the lecithins. A lecithinase in cobra venom can split off an unsaturated fatty acid, producing *lysolecithin*, a substance which has the power of hemolyzing red blood corpuscles. This is the explanation of the toxicity of this venom, as well as of that of certain poisonous spiders and certain other stinging insects. Another lecithinase can split off both fatty acids; another, a phosphatase, can hydrolyze off the phosphoric acid; and still another removes choline from the molecule (Hanahan and Chaikoff). (See page 464.)

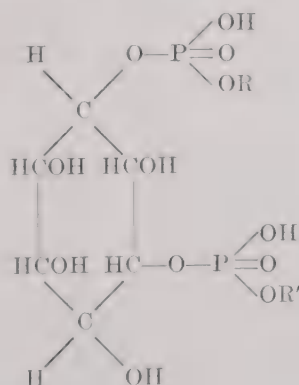
### Cephalins

The cephalins resemble the lecithins in structure except for the component corresponding to choline. This was formerly thought to be ethanolamine in all cases. However, Folch and his associates have shown that brain cephalin is a mixture of phosphatides containing ethanolamine, serine (alpha-amino-beta-hydroxy propionic acid), and inositol. The serine-containing phosphatides seem to be much less in amount than the other phosphatides. (Artom.) They can be separated from one another because of marked differences in solubility in mixtures of chloroform and alcohol. Typical cephalins are:



A cephalin is probably concerned in blood clotting. The cephalins have practically the same solubilities as the lecithins, with one important exception: The cephalins are insoluble in either ethyl or methyl alcohol. They are always associated with lecithins in tissues and most lecithin preparations are really mixtures of these phospholipids.

The inositol phosphatides comprise lipositol and diphosphoinositide. Lipositol was obtained from soybeans. It contains, besides inositol, galactose, oleic acid, and phosphoric acid. (Woolley.) The presence of galactose would seem to relate lipositol also to the glycolipids (see page 93). Diphosphoinositide appears to be made up of equimolecular proportions of inositol metadiphosphate, glycerol, and fatty acid (Folch).



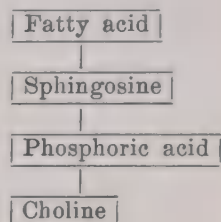
Inositol metadiphosphate

In the above formula R and R' refer to other unknown groups.



### Sphingomyelins

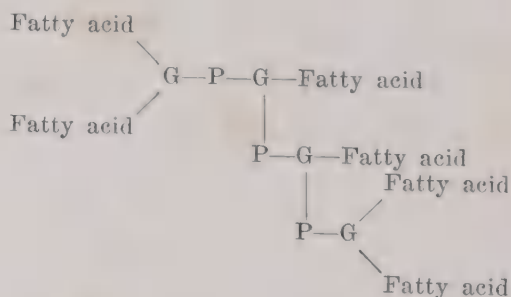
The following scheme will show the probable structure of a sphingomyelin:



Sphingosine is an 18-carbon amine containing two hydroxyl groups:  $\text{CH}_3-(\text{CH}_2)_{12}-\text{CH}=\text{CH}-\text{CHOH}-\text{CHNH}_2-\text{CH}_2\text{OH}$ . Dihydrosphingosine, which is present in some cases, is the fully saturated compound. (Carter and others.) It is evident, therefore, that the sphingomyelins contain two nitrogenous bases, one fatty acid, and one phosphoric acid to each molecule. The fatty acids found in the sphingomyelins of nervous tissue appear to be limited to stearic, lignoceric, and nervonic acids, whereas spleen and lung sphingomyelin contains only palmitic and lignoceric acids. (Thannhauser and Boncoddò.) These lipids are more stable than the other phospholipids. They are not soluble in ether or in cold alcohol but are soluble in chloroform, benzene, and hot alcohol. From hot alcohol they crystallize out on cooling. They rotate the plane of polarized light to the right. Sphingomyelins occur not only in nervous tissue, but also in other tissues.

### Cardiolipins

Pangborn has isolated from heart tissue a new kind of phospholipid, a *cardiolipin*. This contains unsaturated fatty acids and a "polyester" of glycerol and phosphoric acid. The formula might be represented as follows, G representing glycerol and P phosphoric acid:



It has been suggested by Folch-Pi and Sperry that the terms "lecithin," "cephalin," and "sphingomyelin" be abandoned except possibly for crude fractions, because these names have been used indiscriminately to refer sometimes to definite chemical compounds and sometimes to fractions, defined by their solubilities, which consequently may consist of more than one compound. As a result there has been much confusion in the literature. They propose the adoption of the names "phosphoglycerides," "phosphoinositides," and "phosphosphingosides" to designate, of course, those phospholipids containing glycerol, inositol, and sphingosine, respectively. Under this scheme, the phosphoglycerides would include what was formerly called lecithin, i.e., phosphatidyl choline, as well as the "cephalins," phosphatidyl serine and phosphatidyl ethanolamine. Cardiolipins would also belong to this group.

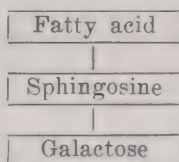
## GLYCOLIPIDS

The glycolipids on hydrolysis yield a sugar, usually galactose, sphingosine, or dihydrosphingosine, and fatty acid. Thus, they contain nitrogen but no phosphorus. Like sphingomyelin, the glycolipids are almost insoluble in ether but they are more soluble in acetone than are the phospholipids and are also soluble in hot alcohol, benzene, and chloroform. The cerebrosides are hydrolyzed by boiling with acids but are more resistant to action by alkalis. No enzymes capable of splitting them have been found.

The glycolipids occur in large amounts in the medullary sheaths of nerves and in brain tissue, particularly in the white matter of the brain, and are often called cerebrosides. They are not found in embryonic brain but develop as medullation progresses. Three glycolipids have been isolated from brain: *kerasin*, *phrenosin*, and *nerrone*. It is believed that they differ only in the individual fatty acids present.

By long hydrolysis with barium hydroxide any cerebroside will yield *psychosin*, which in turn can be hydrolyzed to sphingosine and galactose. Psychosin contains a free amino group and does not reduce alkaline copper solutions. Therefore, galactose is probably linked to sphingosine through its aldehyde group. Since the cerebroside itself does not act as an acid or base and has no free amino group, it is evident that the carboxyl of the fatty acid is attached to the amino group of sphingosine.

Thus a cerebroside, or glycolipid, is built on this plan:



Glycolipid Type Structure

In Gaucher's disease, a congenital and familial derangement, *kerasin* is deposited along with other lipids in the spleen and liver. As much as 10 to 14 per cent of the dry weight of the spleen has been found to be *kerasin* in this condition, and glucose appears to be present in one or more of the cerebrosides isolated. (Klenk and Rennkamp.)

A group of compounds similar to the cerebrosides, and perhaps classifiable with them, is the *gangliosides*, found in the ganglion cells by Klenk. The ganglioside contains one molecule each of stearic acid, sphingosine, and neuraminic acid and three molecules of a monosaccharide. Normally the sugar is galactose; pathologically it may be glucose. Neuraminic acid has the formula  $C_{11}H_{21}NO_8$ .

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## Chapter 5

### PROTEINS

In 1839 Mulder recognized that a large group of important substances had similar general characteristics. These were nitrogenous compounds of biological origin and, at Berzelius' suggestion, since they seemed to be of fundamental importance, he called them *proteins*, from a Greek word meaning "preeminence."

Proteins are probably the most complex of all the known biological substances. They are also probably the most important because, as foods, they are the only sources of the nitrogenous complexes necessary to build protoplasm, and protoplasm itself has as its basis these same compounds, the proteins. Accordingly, we find proteins in all animal and vegetable cells, and many of the enzymes and hormones which accomplish cell activities are proteins. They have been synthesized only by living cells.

Egg white is almost entirely a solution of proteins, and egg yolk, lean meat, fowl, fish, cheese, nuts, legumes, and some cereals are other good sources of protein in our diet. They are, in general, the most expensive foods, as they are the most palatable and essential ones.

Before 1850 the protein materials obtained for study were mixtures of many things besides proteins. About that time, Ritthausen, a German investigator, isolated plant proteins in relatively homogeneous form. He obtained crystalline globulins from many plant seeds. Later, Hoppe-Seyler and Denis purified animal proteins but were unable to achieve the same degree of purity that Ritthausen did. An American biochemist, T. B. Osborne, continued and expanded Ritthausen's protein studies. He prepared and classified many plants and animal proteins. He analyzed them for their constituent amino acids and emphasized the differences in their constitution. In 1905 he predicted, on the basis of their chemical composition, that plant and animal proteins would prove to have different nutritional values.

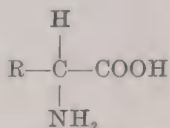
All proteins contain *carbon, hydrogen, oxygen, and nitrogen*; most of them also contain sulfur, and some contain phosphorus. A variety of other elements are present in various proteins, including iron, iodine, copper, manganese, and zinc. The size of the protein molecule is enormous and hence all are colloids, except a few of the simplest decomposition products which are really small fragments of the huge molecule from which they were derived.

The approximate molecular weights of proteins range from 5,000 to 25,000,000. Most of the typical or more common proteins have molecular weights within a somewhat narrower range, perhaps 5,000 to 1,000,000. It is probable that the higher figures will be found to apply to polymers of the proteins, the unit having a considerably lower molecular weight.

In general, protein solutions are opalescent and exhibit the Tyndall phenomenon. Although most proteins are amorphous under ordinary conditions, many have been crystallized; the globulins of many seeds are obtainable in crystalline form, as are the oxyhemoglobins, insulin, many enzymes, and some pituitary hormones.



Decomposition of proteins by hydrolytic agents yields chiefly various alpha-amino acids as final products. This result is obtained no matter what mode of hydrolysis is used—dilute acids, dilute alkalies, or protein-digesting enzymes. Alpha-amino acids have the following type formula:



The R may be a hydrogen, an aliphatic, an aromatic, or a heterocyclic radical. Proteins, therefore, consist entirely, or largely, of combinations of alpha amino acids.

Miller has shown that amino acids are formed when  $\text{CH}_4$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{O}$ , and  $\text{H}_2$  are circulated past an electric discharge. This was done in order to simulate possible primitive earth conditions, which, it has been suggested, postulate an atmosphere of these gases as the precursors of the organic compounds, which serve as the basis of life, rather than  $\text{CO}_2$ ,  $\text{N}_2$ ,  $\text{O}_2$ , and  $\text{H}_2\text{O}$ . The amino acids produced were glycine,  $\alpha$ -alanine,  $\beta$ -alanine, and perhaps others.

## CLASSIFICATION

The different kinds of proteins are classified chiefly on the basis of their solubilities and other physical properties. It cannot be done as yet on the basis of their chemical composition because of the complexity of the protein molecule. First, they may be divided into three general groups: (1) *simple proteins*—protein substances which yield, on complete hydrolysis, chiefly alpha-amino acids, (2) *conjugated or compound proteins*—compounds of a protein with some other molecule or molecules (called the prosthetic group), and (3) *derived proteins*—products formed by the action of heat and other physical forces, or by hydrolytic agents.

The three main groups of proteins are further subdivided.

### I. Simple Proteins

A. **Albumins.**—These are proteins which are soluble in water and in dilute salt solutions and are coagulable by heat. They are precipitated by full saturation of their aqueous solutions with ammonium sulfate. Examples are ovalbumin, serum albumin, and lactalbumin, occurring, respectively, in egg white, blood serum, and milk.

B. **Globulins.**—Globulins are insoluble in water but are soluble in dilute neutral salt solutions; i.e., salts of strong acids with strong bases. They also are heat coagulable. Half saturation with ammonium sulfate is sufficient to precipitate them. The globulins of egg white and of blood serum are examples.

C. **Globins.**—Globins are usually referred to as examples of histones but should be classified separately. They are not basic and are not precipitated by ammonium hydroxide. They are unique in their ability to combine with pigments of the heme class to form hemoglobins.

**D. Prolamins.**—Proteins which are soluble in 70 per cent ethyl alcohol at about the neutral pH are called prolamins. They are high in amide nitrogen and in the amino acid proline. They occur in plants. Examples are gliadin of wheat and zein of corn.

**E. Histones.**—Histones are strongly basic proteins soluble in water but insoluble in ammonium hydroxide. On heating, histones yield a coagulum which is easily soluble in very dilute acids. They are found in cell nuclei as part of the nucleoproteins.

**F. Protamins.**—Protamins are proteins or polypeptides containing relatively small numbers of amino acids. They resemble histones but are soluble in ammonium hydroxide. They occur in cell nuclei.

**G. Albuminoids.**—Albuminoids are characterized by marked insolubility in water and in all neutral solvents. The keratins of epidermal tissue are albuminoids, as are also the elastins, the collagens, and others.

## II. Compound Proteins

**A. Nucleoproteins.**—These are combinations of one or more proteins (histones and protamines) with nucleic acids. They are soluble in dilute NaCl solution and hence may be extracted from tissues by NaCl, and then precipitated by acidification or by dilution with water. Chromatin is a nucleoprotein and viruses are also probably members of this group.

**B. Glycoproteins.**—These are compounds of proteins with a carbohydrate group or with substances, other than nucleic acids, containing carbohydrate groups. Mucin of the saliva is an example. In general, they are slippery substances, and are useful for lubricating or protective purposes.

**C. Phosphoproteins.**—Here the prosthetic group joined to the protein in ester linkage is phosphoric acid. Casein, the principal protein of milk, and vitellin of egg yolk are phosphoproteins elaborated by the maternal organism for the use of the young.

**D. Chromoproteins.**—These are compounds of proteins with heme or some other pigment. Hemoglobins, cytochromes, and flavoproteins are all important members of this group.

**E. Lipoproteins.**—These are simple proteins united with fatty substances. They occur in the blood serum, in brain tissue, eggs, etc.

## III. Derived Proteins

**A. Denatured and Coagulated Proteins.**—When treated with acids or alkalies, or with a variety of other chemical reagents, proteins undergo intramolecular rearrangement. Heat, shaking, or subjection to certain other physical forces have similar effects. The proteins are then said to be denatured and, if a marked lessening of solubility accompanies this, coagulated. The “proteans” and “metaproteins” of the original classification of proteins are simply denatured proteins and do not deserve distinctive names.

**B. Peptides.**—These are hydrolytic cleavage products. As proteins are hydrolyzed either by acid or by enzymes, the huge protein molecules are broken down irregularly. Thus large and small molecules will be broken off, and, as the hydrolysis proceeds, the larger ones will be broken down further and further. Thus, it is easy to see that early in the hydrolysis there will be a comparatively large proportion of the more complex products present and later the mixture will contain simpler and simpler substances. The larger fragments were designated “proteoses” in the early classification, and the smaller ones, “peptones.” Still smaller chains were called “polypeptides” and “peptides.”

“Proteoses” were defined as products soluble in water, uncoagulated by heat, and precipitated by saturating their solutions with ammonium or zinc sulfate. “Peptones” were considered those substances having the same properties as proteoses except that they could not be salted out but could be precipitated by tannic acid. Now it is known that both are *mixtures* of simple amino acids and peptide chains of various lengths. The smaller peptide chains are precipitable by alcohol. The terms “proteoses” and “peptones” are still used commonly for materials employed in the preparation of bacteriological media but are being abandoned by protein chemists.

## OCCURRENCE AND PROPERTIES OF THE PROTEINS

**Albumins.**—These water-soluble proteins are found both in plant and animal tissues. Legumelin in legumes and leucosin in various cereals are examples of plant albumins. Ovalbumin, serum albumin, and lactalbumin have already been mentioned as examples of animal albumins. There are many other animal albumins; e.g., myoalbumin and myogen of muscle, etc. They are heat coagulable; that is, on heating to about 75° C. they are changed to products which are insoluble in water and dilute acid, alkali, and salt solutions. The temperature at which coagulation takes place varies with the protein and also with the pH of the solution.

The albumins may be “salted out” of solution by saturating the solution with ammonium sulfate. In fact, all proteins, except the smaller peptide chains, may be precipitated by this procedure. The addition of various salts to different degrees of saturation precipitates various types of proteins, but full saturation with ammonium sulfate precipitates all, with the exception mentioned. Proteins thus salted out are not coagulated or otherwise changed by this process and may be purified by simply removing the salt used. This may be accomplished by dialysis. Salting out is an excellent method of purifying proteins, including protein hormones and enzymes, prior to crystallization.

**Globulins.**—Globulins also are distributed in both the animal and vegetable kingdoms. There are ovoglobulin and lactoglobulin in bird's eggs and milk, respectively. Blood plasma contains at least four globulins, namely, fibrinogen and alpha, beta, and gamma serum globulins, and muscle tissue contains two or more globulins. Among the plant globulins may be mentioned



edestin from hempseed, which crystallizes rather easily in beautiful octahedral crystals, as well as other crystallizable globulins. These are excelsin from the brazil nut, legumin from legumes, and the globulins from squash and pumpkin seeds. Since globulins are soluble in dilute salt solutions and insoluble in water, their presence in solution in blood and other biological fluids is due to the salts present. If egg white or blood serum is diluted with distilled water, the globulin present will precipitate out, to some extent at least. This method may be used to separate albumins from globulins. Another way is to dialyze such a mixture against water. As the salt dialyzes out, the globulins precipitate.

Since the globulins are less soluble than the albumins, they are more easily salted out. Half saturation with ammonium sulfate is sufficient to precipitate them. This may be done by adding to the protein solution an equal volume of a saturated solution of this salt. Any albumins present will be unaffected. Full saturation with sodium chloride will have the same effect. Thus, albumins and globulins may be separated by selective or fractional salting-out procedures. Each fraction can then be freed of the contaminating salt by dialyzing it against running water or against frequent changes of distilled water. Technique of this sort is utilized in purifying "biologicals," such as diphtheria antitoxin.

Although globulins as a class are coagulable by heat, it must be admitted that vegetable globulins coagulate rather incompletely. In fact many of the type properties of these as well as other classes of proteins have certain exceptions. That is, all the members of a given group do not necessarily possess the same properties in every respect. This is understandable when we realize that the classification is based on a few physical properties rather than on chemical composition.

**Albuminoids.**—The albuminoids are quite insoluble proteins found only in animal tissues. The British classification designates them as *scleroproteins*. The most important members of this group are the keratins, the collagens, and the elastins.

The keratins are the characteristic constituents of epidermal tissue. Horn, hair, nails, wool, hoofs, and feathers—all are chiefly keratin. The outermost layer of the skin is keratin, and a similar albuminoid, keratohyaline, is present in the lower layers. This ultimately is converted into keratin. Albuminoids are insoluble in everything which does not decompose them. This is a most fortunate property. This insolubility of the skin, enhanced by the greasy secretions, protects the surface of the body from water, dilute acid and alkali, and organic solvents. Concentrated acids and alkalies, especially with the aid of heat, will decompose and dissolve keratins. The sulfides of the alkalies and alkali earths will do the same without heat. Various depilatories have as their active ingredient BaS or CaS. It is evident that they must be dangerous to use, since they act not only on the keratin of the hair, but also on that of the skin, tending to denude or, at least, to injure it. All hard keratins, on hydrolysis, yield, as part of their amino acid constituents, histidine, lysine, and arginine in definite molecular



proportions; namely, in the ratio 1:4:12. The "soft" or pseudokeratins, such as those occurring in the outermost layers of the skin, do not have these three amino acids in quite the same proportions. In "neurokeratin" (see page 141), however, the ratio is 1:2:2, indicating that this nerve protein is not a true keratin.

Keratin has been considered indigestible, but this statement must be modified since it has been shown that if it is pulverized to a very high degree, it can be digested by appropriate enzymes. If ingested as found in nature, however, no digestion occurs. Clothes moths and many microorganisms possess enzymes which can digest keratin.

Collagen is found in connective tissues and also in bone. Like keratin, it is insoluble in all neutral solvents. It is converted into a very tough, hard substance on treatment with tannic acid. This is the basis of the tanning process. The most interesting property of collagen is its ready conversion to gelatin. Since this conversion involves the splitting off of some amino acids from the molecule, gelatin must be an intermediate hydrolysis product. Collagen is thus changed from an insoluble to a highly soluble product and from a very slowly digestible one to one that is readily digested. Gelatin forms a gel on cooling, and this property is highly esteemed in dietetics. It is a favorite article of diet for invalids and convalescents because of its palatability and ease of digestion. It should be pointed out, however, that it is by no means a complete protein and should not be the sole or main protein in any diet continued over a long period of time. In certain pathological conditions, such as cirrhosis of the liver, the tissue acquires an increased content of collagen.

Elastins are the proteins of the yellow elastic fibers of connective tissues. The ligamentum nuchae is almost pure elastin. Elastins differ from the collagens in not being converted to gelatins. They are, however, rather easily digested by pepsin and by trypsin.

**Globins.**—These proteins, which unite with heme and similar pigments to form hemoglobin, etc., are not basic in water solution, although they have a high content of a basic amino acid, histidine. In fact, their isoelectric points are in the neighborhood of pII 7.0. (See page 111.) A compound of a globin with insulin has recently been prepared for clinical use.

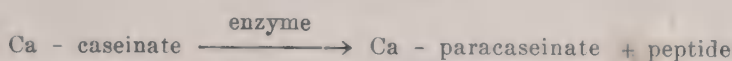
**Prolamins.**—These alcohol-soluble proteins are found chiefly in cereals. About half of the total protein of white flour (wheat) is gliadin, a prolamins. This is a nutritionally deficient protein because of its lack of lysine, one of the essential amino acids. For this reason white flour is a poor source of protein. Corn contains zein, and barley, hordein, both prolamins. Both are inadequate proteins since zein is low in lysine and tryptophan, and hordein is low in lysine and valine; all three are indispensable amino acids.

**Histones.**—Histones occur in highest concentration in those tissues in which large numbers of nuclei are found. They are rich in arginine. When injected into animals, histones may have toxic effects, such as causing the blood to clot less readily.

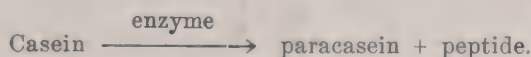
**Phosphoproteins.**—Casein of milk and vitellin of egg yolk are the most important phosphoproteins. They are compounds of proteins and phosphoric acid. Casein is present in milk as the calcium salt, calcium caseinate. The precipitation of casein from milk may be accomplished in two ways. Acidification of the protein to its isoelectric point causes it to precipitate.



The action of proteolytic enzymes *apparently* has the same effect. However, this is not quite true because a partial digestion takes place, some fragment of the protein molecule being split off. It further differs from acid precipitation in that the precipitate contains calcium. In fact, in the absence of calcium the precipitation does not occur. Thus:



but



The paracasein remains in solution until Ca ions are added:



Under suitable conditions almost any proteolytic enzyme will cause milk to clot in this way, but rennin, the enzyme present in the fourth stomach of the calf, is especially effective and apparently its action is limited to this digestion.

**Nucleoproteins.**—Nucleoproteins are compounds of ribose or deoxyribose nucleic acids with proteins of different degrees of basicity. The chromatin of the nucleus, and, hence, of the chromosomes, contains predominantly deoxyribose nucleic acids, linked to basic proteins, such as the protamins of certain fish sperms, or histones. The solubility and stability of these complexes vary from species to species and in various types of tissues. Thus, the nucleohistone of calf thymus may be extracted with distilled water, while the corresponding nucleoprotein in nucleated erythrocytes of birds is insoluble in distilled water. All nuclear nucleoproteins are insoluble at the ionic strength corresponding to the salt concentration which is "physiological" for the particular species; i.e., 0.14 M NaCl in the instance of mammals and 0.28 M in the case of certain marine fishes. On the other hand, they are soluble in strong neutral salt solutions (M NaCl). However, in this solvent the bond between the nucleic acid and the protein fractions is either greatly weakened or completely dissociated. The precipitate obtained by dilution of such a solution with water represents an artifact, a so-called "protein nucleinate." According to all indications, the linkage is saltlike in character; i.e., ionic. At low ionic strength, the nucleoproteins from liver and thymus will migrate with a single boundary in the electrophoresis apparatus. (See page 113.) The molecular weight of thymus nucleohistone is of the order of two million; that of thymus nucleic acid alone, of the order of one million. Since the molecular weight of pro-

tamins is about 5,000 and of histones about 30,000, this indicates that many protein residues are combined with a single nucleic acid molecule in complexes of this type.

The ribose nucleic acids occur chiefly in the cytoplasm, where they are combined with proteins and lipids, and are organized in the form of discrete, microscopic, and ultramicroscopic granules called microsomes. The bond between ribose nucleic acids and proteins is, as a rule, stronger than that between desoxyribose nucleic acids and the more basic proteins. The molecular weights of these complexes are not known.

**Glycoproteins.**—Glycoproteins have, as their prosthetic groups, complex polysaccharides. A typical glycoprotein, tendomucoid, yields on hydrolysis a protein and chondroitic acid. The latter is a complex compound which on further hydrolysis is broken up into sulfuric acid, acetic acid, galactosamine, and glucuronic acid. Other glycoproteins are the mucins of saliva, of the gastrointestinal "mucus," of the ocular and synovial fluids, and the blood-group A factor of the gastric mucosa. They are slippery and hence have lubricating value. The carbohydrates of the mucins are glucosamine or some of the uronic acids. One of the latter is hyaluronic acid, a complex consisting of N-acetyl-D-glucosamine and D-glycuronic acid. Hyaluronic acid is found in the capsules of certain strains of pneumococci, streptococci, and other microorganisms, as well as in the glycoproteins of the umbilical cord, the vitreous humor, and the synovial fluid. Other proteins have small amounts of carbohydrates present as actual molecular components. The consensus of opinion is to consider as glycoproteins only those which have over 10 per cent of carbohydrate. Such a proportion would show that the carbohydrate is a characteristic prosthetic group.

Glycoproteins are not heat coagulable. They are not digested by the gastrointestinal enzymes, and for that reason their presence in the slimy secretions is protective. They are precipitated out of aqueous solutions by acidification.

### General Properties of Proteins

1. Proteins have a distinctive odor when burned—the "odor of burning hair."
2. They are precipitated by salts of the heavy metals; e.g., mercury, silver, lead. This occurs on the alkaline side of the isoelectric point. (See page 111.) The metals unite with the carboxyl groups, thus forming mercury proteinates, etc. The use of silver nitrate in cauteries is based on this property; it precipitates the proteins of tissues as silver salts. Another application of this property is the use of proteins as antidotes to metallic poisons. Egg white, milk, and other liquid protein can be used, but the metallic protein precipitate must be removed from the stomach by an emetic or by a stomach tube to prevent the digestion of the protein and the liberation, re-solution, and absorption of the poisonous metal.
3. Certain acids precipitate proteins. Here the proteins react because of their possession of free  $\text{NH}_2$  groups. The acids which precipitate the proteins in this way are sometimes termed "alkaloidal reagents" because



they precipitate many of the alkaloids. Included among these are trichloroacetic acid, phosphotungstic acid, and phosphomolybdic acid, yielding protein trichloroacetates, phosphotungstates, and phosphomolybdates. Perchloric acid has recently been introduced as an effective protein precipitant (Neuberg and co-workers). Another one, sulfosalicylic acid, is used to detect proteins qualitatively and determine them quantitatively in urine and body fluids. Tannic acid is used commercially to precipitate collagen in hides, thus yielding leather.

4. Proteins unite with certain dyestuffs. Among these are picric acid and flavianic acid, but there are many others, as the variously dyed woollens indicate.

5. Proteins give certain color tests. These reactions are not specific for the protein molecule as such but for the characteristic group of particular amino acids contained in the protein.

a. *Xanthoproteic Reaction*.—Concentrated nitric acid turns proteins yellow. Neutralization with sodium hydroxide deepens this to an orange. This test, which will be recognized as the nitric acid stain produced on the skin, is due to the nitration of the benzene nucleus present in the aromatic amino acids; e.g., phenylalanine, tyrosine, and tryptophan.

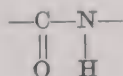
b. *Millon's Test*. Millon's reagent consists of mercurous nitrate in nitric acid. If a protein is treated with Millon's reagent and heat is applied, a red color is produced. Any phenolic compound will give this reaction and, therefore, compounds containing tyrosine will react positively to it.

c. *Sakaguchi Reaction*. This test consists in treating an alkaline solution with alpha naphthol and sodium hypochlorite. A red color develops if substituted guanidines are present. It is accordingly a test for arginine or proteins containing arginine.

d. *Nitroprusside Reaction*. Proteins containing cysteine give a reddish color with sodium nitroprusside in dilute ammoniacal solution.

e. *Test for -SH Groups*. Another test for cysteine is to boil the solution with sodium hydroxide. The sulfur splits off to form  $\text{Na}_2\text{S}$ . This should be cooled, acidified, and then heated again.  $\text{H}_2\text{S}$  will come off and will blacken lead acetate paper held over the mouth of the test tube.

f. *Biuret Test*. The solution to be tested is made strongly alkaline with sodium hydroxide and is then treated with a small amount of very dilute copper sulfate solution. A rose-pink to violet to purple is produced. The deeper the color, the more complex the protein. The color depends upon the presence of two or more peptide linkages, that is, linkages of this type,



joined to each other or separated by a single atom of carbon or nitrogen. Consequently, free amino acids will not give the biuret test. Other closely related groups give similar colors. For example,



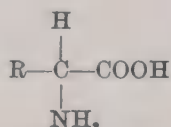


Ammonium salts interfere with this test by forming a deep blue compound. Therefore, if a large amount of ammonium sulfate is present, as may be the case in salting-out operations, a great excess of alkali must be employed.

g. *Ninhydrin Reaction*. (See page 109.)

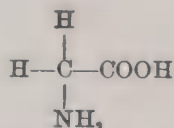
## AMINO ACIDS

There are about twenty amino acids in the more common plant and animal proteins. All are alpha-amino (or else imino) acids having the following general formula:

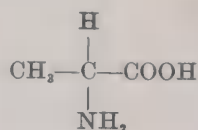


It will be remembered that the alpha-carbon of an organic acid is that carbon nearest the carboxyl; the beta carbon is second, etc. The names and formulas of the more important amino acids follow:

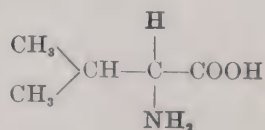
1. Glycine, or glycocoll, amino acetic acid.



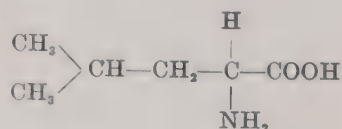
2. Alanine,  $\alpha$ -amino propionic acid.



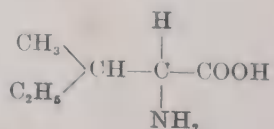
3. Valine,  $\alpha$ -amino isovaleric acid.



4. Leucine,  $\alpha$ -amino isocaproic acid.

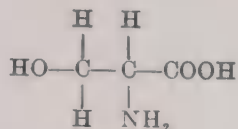


5. Isoleucine,  $\alpha$ -amino  $\beta$ -methyl- $\beta$ -ethyl-propionic acid.

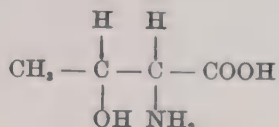


The above are all comparatively simple mono-amino, mono-carboxylic acids. The next two are closely related hydroxy acids.

6. Serine,  $\alpha$ -amino  $\beta$ -hydroxy propionic acid.

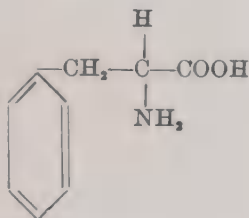


7. Threonine,  $\alpha$ -amino  $\beta$ -hydroxy butyric acid.

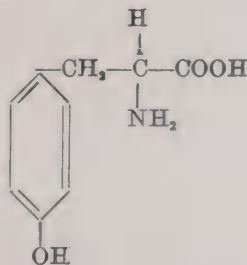


Two mono-amino, mono-carboxylic acids with aromatic groups are phenylalanine and tyrosine.

8. Phenylalanine,  $\alpha$ -amino  $\beta$ -phenyl propionic acid.

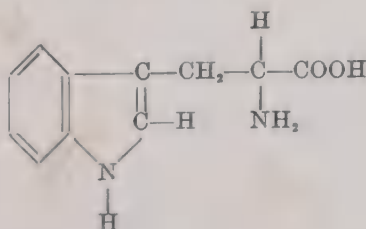


9. Tyrosine,  $\alpha$ -amino  $\beta$ -*p*-hydroxyphenyl propionic acid (*p*-hydroxyphenyl alanine).



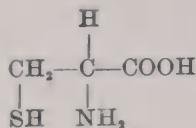
An amino acid of the same series but containing a heterocyclic group is tryptophan.

10. Tryptophan,  $\alpha$ -amino  $\beta$ -indole propionic acid.

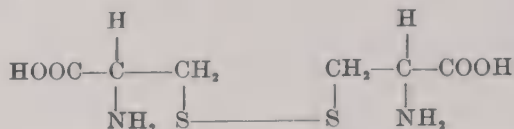


Two mono-amino mono-carboxylic acids contain sulfur. They are cysteine and methionine. A third sulfur-containing amino acid is cystine, which is dicysteine.

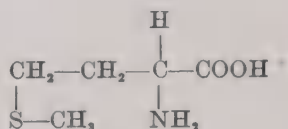
11. Cysteine,  $\alpha$ -amino  $\beta$ -thio propionic acid.



12. Cystine, di-cysteine.

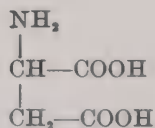


13. Methionine,  $\alpha$ -amino  $\gamma$ -methyl-thiol butyric acid.

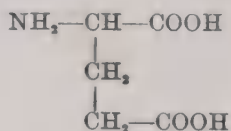


Two amino acids, each of which has two carboxyl groups, are aspartic acid and glutamic acid. It is these dicarboxylic acids which give proteins some acidic characteristics.

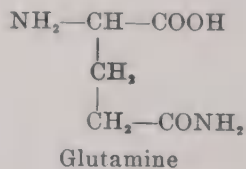
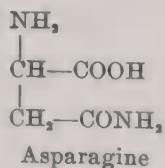
14. Aspartic acid, amino succinic acid.



15. Glutamic acid,  $\alpha$ -amino glutaric acid.

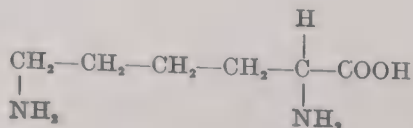


Aspartic acid and glutamic acid probably occur in proteins largely as the corresponding acid amides, asparagine and glutamine:



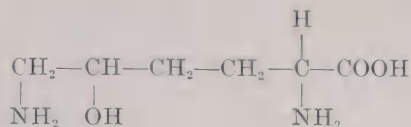
Of the basic amino acids, that is, those having two amino groups or one amino and one imino group, the following are of importance:

16. Lysine,  $\alpha$ - $\epsilon$ -diamino caproic acid.

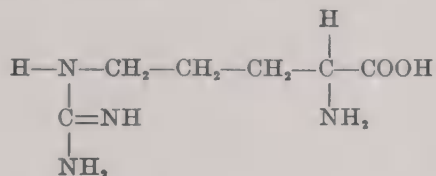




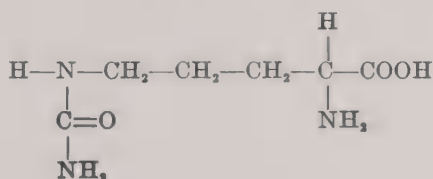
17. Hydroxylysine,  $\alpha$ - $\epsilon$ -diamino- $\delta$ -hydroxy caproic acid.



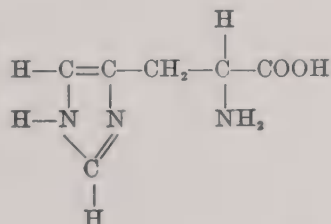
18. Arginine,  $\alpha$ -amino  $\delta$ -guanido valeric acid.



19. Citrulline,  $\alpha$ -amino  $\delta$ -carbamido valeric acid.

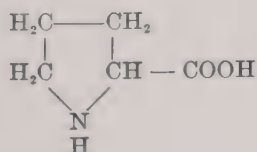


20. Histidine,  $\alpha$ -amino  $\beta$ -imidazol propionic acid.

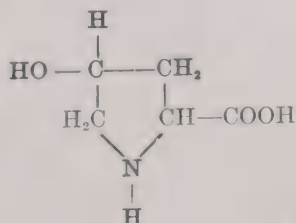


Finally we have two pyrrolidine derivatives, in which the nitrogen of the imino group is in a ring but can still function in the formation of peptides.

21. Proline, pyrrolidine- $\alpha$ -carboxylic acid.



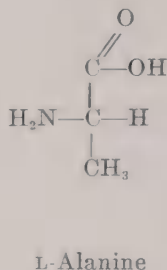
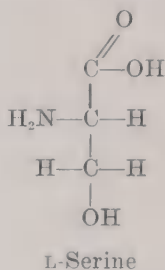
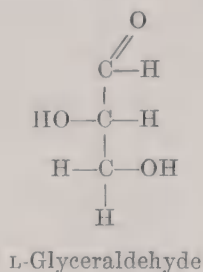
22. Hydroxyproline,  $\gamma$ -hydroxypyrrolidine- $\alpha$ -carboxylic acid.



In addition to these amino acids which are commonly found in proteins, it may be mentioned that the thyroid gland has the ability to iodinate tyrosine,

producing 3-iodo tyrosine and 3,5-diodo tyrosine. These are used by the thyroid gland to produce triiodothyronine and thyroxine, one or both of which may be the thyroid hormone or hormones. (See page 615.) All four compounds are  $\alpha$ -amino acids.

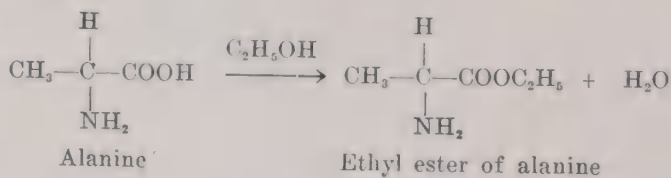
Examination of the formulas of the above amino acids will reveal the fact that all, except glycine, have an asymmetric carbon. It is therefore possible to have a D-form and an L-form of each. Most of the naturally occurring  $\alpha$ -amino acids are of the L-form, that is, their configuration is similar to that of L-glyceraldehyde, or, more appropriately, to L-serine.



All of the formulas of the amino acids can be written in this way, but since the L configuration is the common one, we shall usually omit the letter L in connection with the amino acids. Apparently the enzymes of the body can split chiefly those peptides composed of L-amino acids; that is, the normal ones. Certain bacteria are enclosed in capsules composed of D-amino acid combinations. Consequently, when ingested they escape digestion and can gain entrance to the body because of this protection. It has been claimed that cancer tissues contain D-amino acids in their proteins. This has not been definitely established, however. Again it should be emphasized that D- and L- do not refer to dextro- or levorotation but to the relationship to D- and L-serine, respectively.

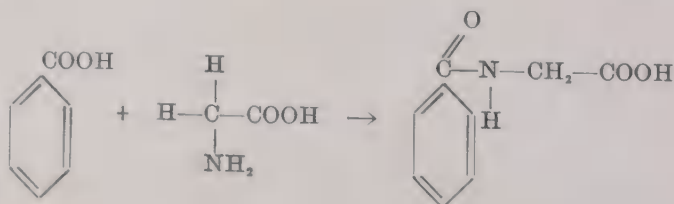
### Reactions of Amino Acids.—

1. All amino acids can be esterified.



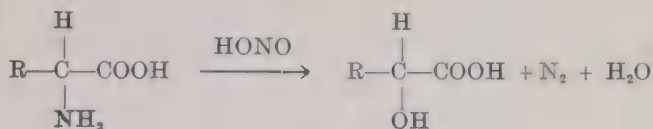
This property was made use of by Fisher, who separated amino acids from each other by fractionally distilling their esters.

2. Amino acids will acetylate and benzoylate. Thus, glycine will combine with benzoic acid.



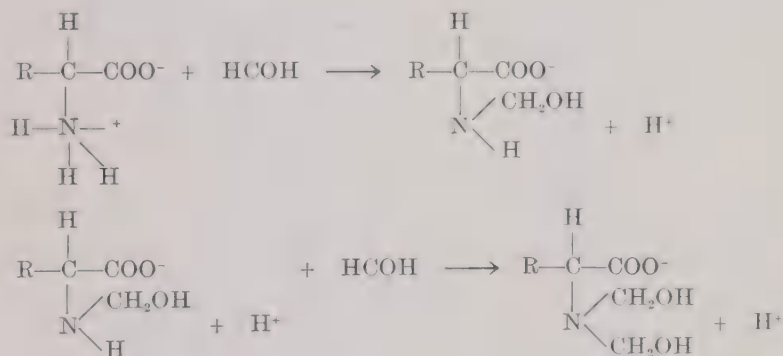
This reaction occurs in the body when benzoates are ingested.

3. Like other primary amines, the amino acids (except proline and hydroxy-proline, which are really imino acids) react with nitrous acid, liberating nitrogen gas.



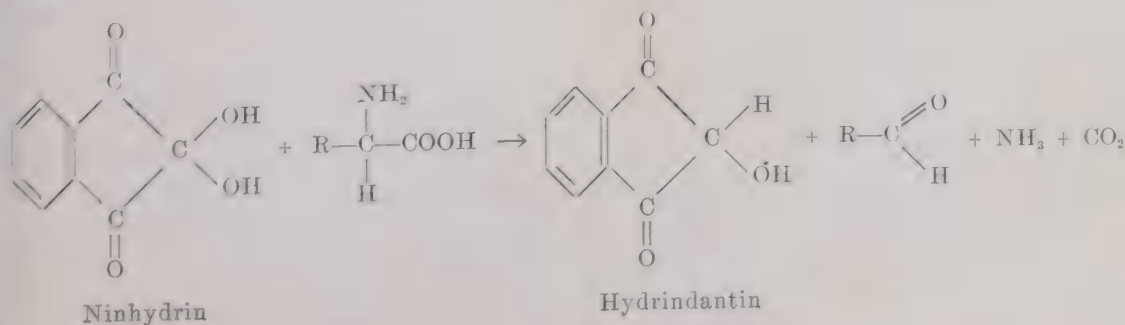
This is the basis for the Van Slyke method for determining amino groups in proteins, blood, and other biological material.

4. Formaldehyde reaction. The addition of an excess of neutral formaldehyde solution to a *neutral* amino acid solution results in a distinctly acid mixture. The amino acid is completely ionized (see page 112) and the reaction is, probably:



This is the Sørensen reaction. By titration with standard alkali, the amount of free amino acid present can be estimated. This is often made use of in following the course of a protein digestion in which amino acids are liberated. It is evident that this is really a measure of the amount of amino groups present.

5. Reaction with ninhydrin. If a solution of an alpha-amino acid is boiled with ninhydrin (triketohydrindene hydrate)  $\text{CO}_2$  is split off and a purple color is produced. All alpha-amino acids give this reaction, as do the proteins and peptides. Proline and hydroxy-proline give a yellow color and the various other amino acids give slightly different shades as well as depths of color. In the course of the reaction the carboxyl of the amino acid is quantitatively converted into  $\text{CO}_2$ , which can easily be determined. This has been utilized in a method for the quantitative determination of amino acids (Van Slyke).

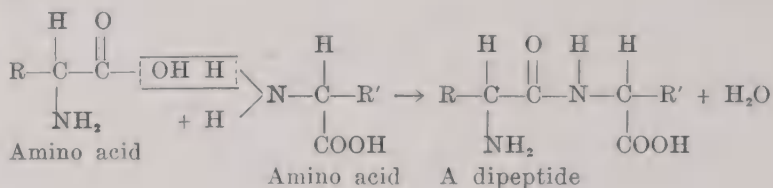


The bluish color which results is due to the formation of a complex by the union of ninhydrin, hydrindantin, and ammonia.

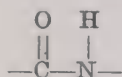
6. Color reactions. Most of the color reactions of the proteins are referable to individual amino acids in their molecules which give these characteristic tests, as has been mentioned under General Properties of the Proteins. (See page 103.)

### PEPTIDE LINKAGE

In 1902 Fischer and Hofmeister, independently, proposed the peptide hypothesis of protein structure. This postulates that the amino acids are joined by the union of carboxyl groups to amino groups, with the elimination of a molecule of water at each linkage. Thus a dipeptide is formed from two amino acids in this way.



A third amino acid will form a tripeptide by being linked through its carboxyl to the  $\text{NH}_2$  of the first amino acid, or through its  $\text{NH}_2$  group to the carboxyl of the second. The grouping which joins the alpha-amino acids together in this way



is called the *peptide linkage*. Peptides varying in length from the simplest dipeptide to very long chains have been synthesized and have some protein properties.

**POLYPEPTIDE ANTIBIOTICS.**—Some of the antibiotics (see page 668) have proved to be of polypeptide nature. Gramicidin and tyrocidin, produced by *B. brevis*, are bactericidal substances, discovered by Hotchkiss and Dubos. They yield, on hydrolysis, a variety of amino acids, some of which are D-amino acids. Whether the D-forms are responsible for the bactericidal action of these compounds is not known, but their presence is significant. They are not single polypeptides, as was thought at first, but are mixtures. Bacitracin, produced by *B. subtilis* (Tracy), is another antibiotic composed of one or more polypeptides (Craig).

The method of naming polypeptides is shown in the example on the opposite page. Note that the amino acid having the free end carboxyl is named last.

The evidence in favor of the hypothesis that the peptide linkage is the principal type of union in proteins has been summarized by Vickery and Osborne in part as follows:

1. Native proteins contain very little free amino nitrogen.



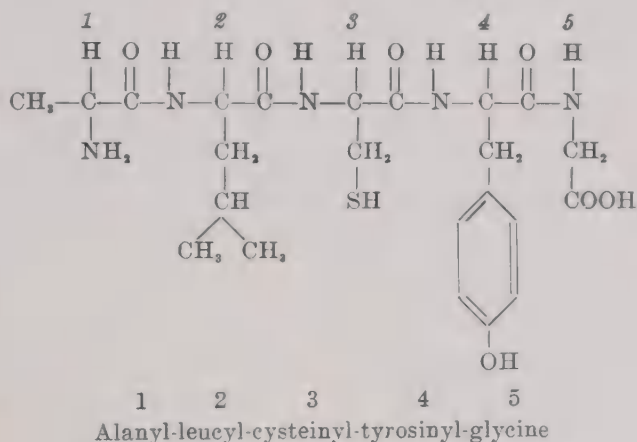
2. The biuret reaction, given by proteins, is also given by other substances having a peptide or similar linkage. However, when a protein is hydrolyzed, the biuret reaction becomes progressively weaker until finally, on complete hydrolysis, it disappears.

3. The synthetic polypeptides prepared by Fischer and others can be hydrolyzed by the same enzymes which hydrolyze native proteins.

4. Polypeptides have been isolated from incomplete protein digests.

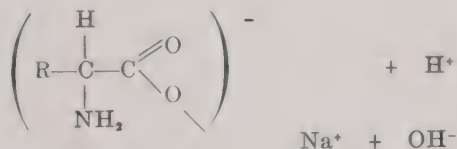
5. During acid or enzyme hydrolysis of proteins, amino groups and carboxyl groups are liberated progressively at about the same rate.

6. During such hydrolysis there is very little change in reaction, indicating that acidic and basic groups are released in approximately equivalent amounts.

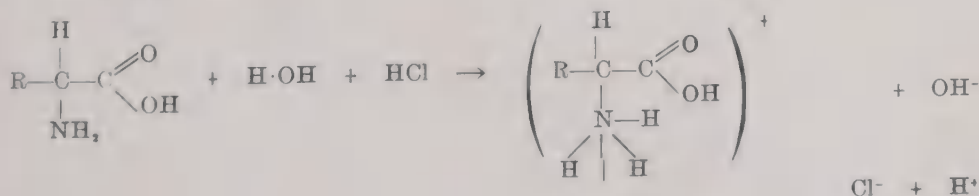


### Isoelectric Point

A typical amino acid possesses an amino group and a carboxyl group and consequently may be assumed to act either as a base or an acid. In alkaline solution it behaves like an acid:



If an electric current is passed through such a solution, the amino acid anion will migrate to the anode or positive pole. In acid solution, on the other hand, it behaves like a base:

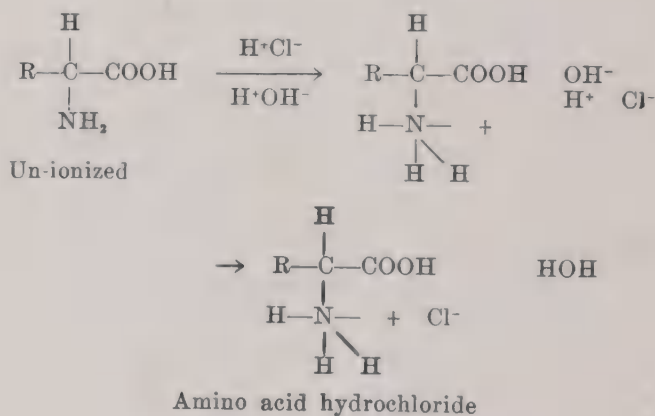


The passage of an electric current under these conditions will cause the amino acid cation to go to the cathode or negative pole. If we should change the

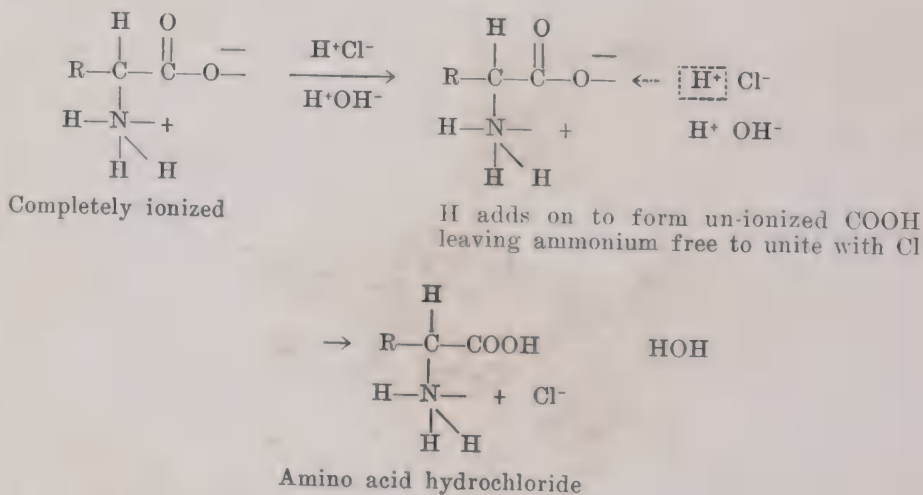
reaction of the alkaline solution, making it less and less alkaline, or make the acid solution less and less acid, it is evident that at some point a degree of acidity or alkalinity would be reached at which the amino acid would travel in neither direction under the influence of the electric current. That hydrogen ion concentration is called the "isoelectric point." At the isoelectric point the electric charges present on the amino acid balance each other. This point may or may not be at the neutral figure, pH 7.0, as may be seen from Table VIII.

The former view that an amino acid is un-ionized at its isoelectric point is now replaced by the concept that it is *completely ionized*. The positive charge and negative charge thus formed neutralize each other entirely. The old or classical view and also the modern conception of the dipolar ("zwitterion") form are shown below. First, let us consider the amino acid in an acid solution.

Classical:



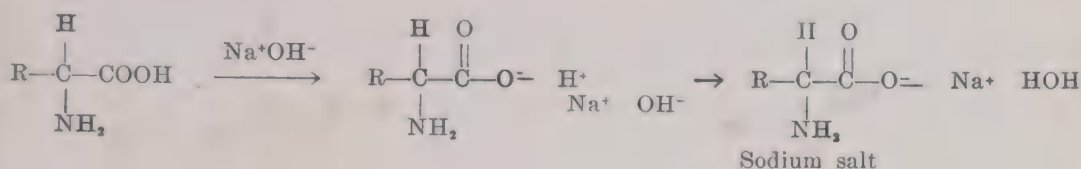
Dipolar:



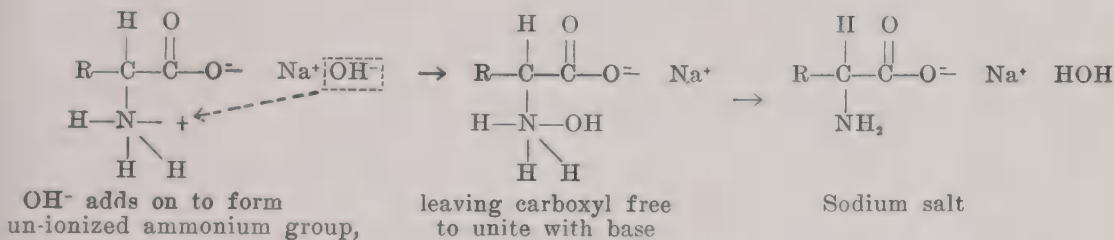
The result is the same in both cases, but the mechanism is different. According to the classical theory, adding acid permits ionization of the amino group, whereas the newer theory maintains that addition of acid depresses the ionization of the carboxyl and leaves the ammonium group free to unite with the negative ion.

In alkaline solution two similar sets of reactions may be shown:

Classical:



Dipolar:



Again we have the same result, but here the dipolar theory indicates a depression of the amino ionization, leaving the carboxyl free. The dipolar theory is more in accordance with the physicochemical behavior of the amino acids in a number of ways.

Proteins are compounds of many amino acids joined in peptide linkages. They are certain to have an excess of both carboxyl and amino groups (because of the presence of both acidic and basic amino acids). Since these free carboxyl and amino groups are jutting out from the molecule, a protein may be regarded as a complex amino acid. For this reason proteins have isoelectric points. At the isoelectric point of a protein the positive and negative electric charges are balanced. Furthermore, although usually a protein is in solution at its isoelectric point, it is least soluble at that point and consequently is most easily precipitated. This is also the point of lowest viscosity and swelling. Proteins differ greatly in their isoelectric points, as is indicated in Table VII. A knowledge of the isoelectric point of a given protein is extremely useful. In the preparation and purification of sera, hormones, etc., for therapeutic purposes, advantage is taken of isoelectric points, and in other ways this factor is frequently made use of.

**Electrophoresis.**—The movement of a charged particle in an external electrical field toward the oppositely charged electrode is called electrophoresis. The electrophoresis of biocolloids, for the most part proteins, has been extensively studied during the past decade.

The method commonly employed depends upon optical observations of a moving boundary. The technique and apparatus were developed by Tiselius and his co-workers in Sweden, and by Longsworth, Stern and others in this country. Current is applied to the ends of a U tube which is filled with a protein solution overlaid by a buffer solution of the same ionic strength, pH, and conductivity. The buffer solution is stratified carefully over the protein layer so that the boundary between the two solutions is very sharp. During the electrophoresis

TABLE VII  
ISOELECTRIC POINTS OF SOME PROTEINS\*

PROTEIN	SOURCE	ISOELECTRIC POINT pH
<i>Recrystallized Proteins</i>		
Edestin	Hempseed	5.5-6.0
Egg albumin	Hen's egg	4.55-4.90
Hemocyanin	Snail blood	5.05
Hemoglobin, reduced	Horse blood	6.79-6.83
Hemoglobin, oxy-	Horse blood	6.7
Insulin	Beef pancreas	5.30-5.35
Lactoglobulin	Cow's milk	4.5-5.5
Serum albumin	Horse blood	4.88
Trypsin	Beef pancreas	5.0-8.0
Urease	Jack bean	5.0-5.1
<i>Amorphous Proteins</i>		
Bence-Jones	Human urine (pathol.)	5.20
Casein	Cow's milk	4.6
Gelatin	Calf's skin	4.80-4.85
Gliadin	Wheat (flour)	6.5
Myogen	Rabbit muscle	6.2-6.4
Myosin	Rabbit and cow muscle	6.2-6.6
Protamines	Fish sperm (from 9 different kinds of fish)	12.0-12.4
Protamines	Fish sperm (3 other varieties)	9.7, 10.0, 11.7
Serum globulin	Horse blood	5.4-5.5
Silk fibroin	Silk	2.0-2.4

\*From Schmidt, C. L. A.: The Chemistry of the Amino Acids and Proteins, Springfield, Ill., 1938, Charles C Thomas. Corrected according to Addendum, 1943.

TABLE VIII  
ISOELECTRIC POINTS OF AMINO ACIDS AND PEPTIDES AT 25° C.\*

SUBSTANCE	ISOELECTRIC POINT pH	SUBSTANCE	ISOELECTRIC POINT pH
Alanine	6.1	Phenylalanine	5.9
Arginine	10.8	Proline	6.4
Aspartic acid	3.0	Serine	5.7
Cysteine (30° C.)	5.1	Tryptophan	5.9
Cystine (30° C.)	5.6	Tyrosine	5.7
Diiodotyrosine	4.3	Valine	6.0
Glutamic acid	3.2		
Glycine	6.1	Aspartyl-aspartic acid	3.0
Histidine	7.6	Alanyl-alanine	5.8
Beta-hydroxyglutamic acid	3.3	Glycyl-alanine	5.7
Hydroxyproline	5.8	Glycyl-glycine	5.6
Isoleucine	6.0	Glycyl-glycyl-glycine	5.6
Leucine	6.0	Glycyl-alanyl-alanyl-glycine	5.6
Lysine	9.7	Histidyl-histidine	7.3
Methionine	5.8		
Norleucine	6.1		

\*From Schmidt, C. L. A.: The Chemistry of the Amino Acids and Proteins, Springfield, Ill., 1938, Charles C Thomas.



period the various proteins which were originally present at the boundary between the buffer and the protein solution become separated, the degree depending on the magnitude of their charges. Several new boundaries may be formed, if more than one protein is present, and their locations in the limbs of the tube (and therefore the distances traveled from the starting position), as well as their relative concentrations, may be determined by observing the extent to which light is refracted by the various boundaries. This is accomplished by complex optical systems, the descriptions of which are beyond the scope of this book. The type of pattern obtained is shown in Fig. 18, page 172.

The Tiselius apparatus has found numerous applications. It is used to determine the isoelectric point and the electrophoretic mobility of proteins. Diffusion constants (which, together with sedimentation velocities obtained by ultracentrifugation, enable one to calculate the molecular weight of a protein) may be measured in the Tiselius apparatus. Studies of the undenatured proteins of organ extracts have been carried out by moving boundary methods in an attempt to characterize the various protein complexes. The method is also frequently employed to study the homogeneity of a protein; that is, whether a given material consists of one or more entities. Finally, it is possible to isolate specific proteins from mixtures, a method which has obvious advantages over separation by salting-out procedures or chemical fractionation. Clinically, the study of plasma proteins and organ proteins in health and disease by electrophoresis has become a very active field of research.

### Denaturation and Coagulation

Albumins and globulins in aqueous solution, at or near their isoelectric points, are rendered insoluble on heating. The same is true of hemoglobins. This phenomenon is known as coagulation. Although coagulated proteins may be redispersed or redissolved, this requires special conditions and the protein is not of the same nature as the original uncoagulated protein. For all practical purposes, therefore, we may say that coagulation renders the protein insoluble, and the process is irreversible. Individual proteins have more or less characteristic "coagulation points," which may be altered by changing the pH slightly. The most favorable pH for coagulation coincides with its isoelectric point (see page 111); i.e., coagulation occurs at the lowest temperature at this point. At other pH's, coagulation is delayed or occurs at a higher temperature. Coagulation in all cases is preceded by *denaturation*. This involves an intramolecular rearrangement which, to some extent, is reversible. Denatured proteins are soluble in dilute acid or alkali but are insoluble at the isoelectric point. Precipitation at this point is called *flocculation*, and the *flocculum* is soluble on either side of the isoelectric point, if this is accomplished without delay. If, however, there is a delay, or if heat is applied, the denatured protein is changed from a flocculum to a coagulum, which is not soluble in dilute acid, in dilute alkali, or at its isoelectric point.

Proteins, other than albumins and globulins, may be denatured even though they are not heat coagulable. Other methods of denaturation besides heating

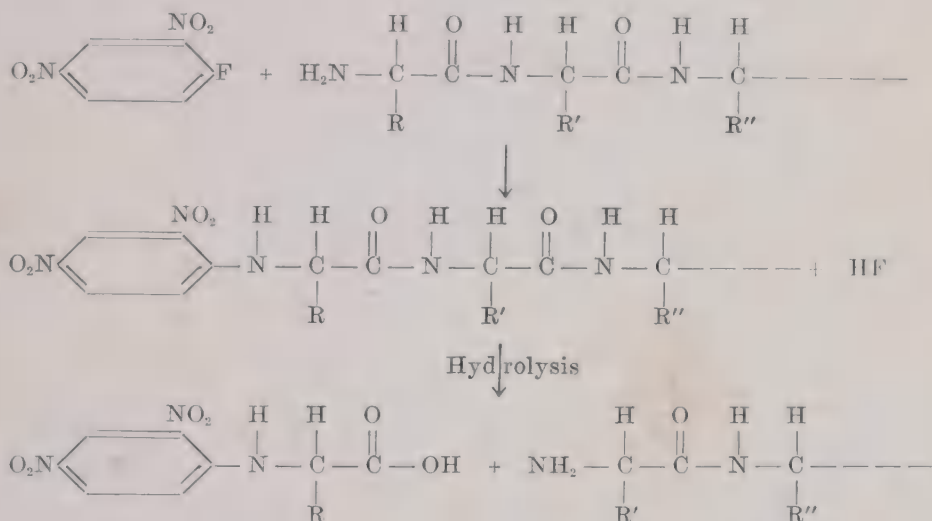
include action of strong acids or alkalis, salts of the heavy metals, light, mechanical agitation, alcohol, acetone, ether, urea, and the detergents. Water is necessary for denaturation and coagulation by heat, as can be shown by heating dry egg white to over 100° C. without loss of solubility. Denaturation involves a structural change in the protein molecule, but there is apparently no hydrolysis. There results a change in viscosity, a lower surface tension and, in the case of hemoglobins, a loss of species specificity.

Denatured proteins are attacked by digestive enzymes differently from undenatured proteins. For example, denatured albumin and crystalline albumin are digested at the same rate by pepsin, but trypsin digests the former much more rapidly. The effect of light as a denaturing agent is seen in the clouding of the lens of the eye in old-age cataract. This is probably due to the denaturing of the globulins present in the lens. Glass workers are particularly subject to cataract, presumably as a result of the infrared rays emanating from the molten glass. The change of fibrinogen to fibrin in blood clotting is no doubt a denaturation reaction.

**Structure of Protein Molecule.**—The amino acids appear to be joined together in the protein molecule by peptide linkages—probably *only* by peptide linkages. The problem of how these chains of peptides are arranged in the molecule remains to be solved. Several complex hypotheses have been put forward with more or less basis in each case. It is known that several different arrangements of long chains occur. In the chains, the amino acids are arranged in complex linear patterns. Different proteins contain different proportions of their constituent amino acids, with a special arrangement or pattern for each protein. The chemical, physical, and biological properties of each protein are set by this amino acid pattern. The simplest is a long straight chain. Silk fibroin is the best example. Evidence that it is a single chain is found in the fact that it cannot be stretched; in fact, silk fibroin has the same tensile strength as the best steel of the same diameter. There is x-ray evidence tending in the same direction. Wool protein, a keratin, has its peptide chains folded. It can be stretched, that is, unfolded, and can be shrunk, which means that it is folded still more. Hair keratin is of a similar nature. It, too, can be stretched. When this is done under the influence of suitable reagents, the hair strands may be stretched as desired, resulting in a “permanent wave.” These are examples of fibrous proteins. “Globular” proteins have their peptide chains folded in a different fashion, perhaps more like coils. When they are uncoiled, denaturation takes place and it is difficult, but not always impossible, to get them coiled again. In all except the single straight chains we can imagine bridges holding the chains together. These may be —S—S— linkages, unions between  $\text{NH}_2$  and  $\text{COOH}$  groups (from basic and acid amino acid units, respectively), or residual or secondary valencies. The breaking of these bridges probably also contributes to denaturation and coagulation, and perhaps to digestion of proteins.

**The Structure of Insulin.**—Insulin, the hormone produced by the islands of Langerhans of the pancreas, is a protein and has been studied very in-

tensively. Its molecular weight has been shown to be about 6,000 (Harfenist and Craig). By ingenious new techniques, the structure of insulin has been almost completely worked out by Sanger and associates. The reagent, dinitrofluorobenzene, combines with the alpha-amino acids at the ends of peptide chains. When a peptide, which has been so treated, is hydrolyzed, the dinitrophenyl groups (DNP) remain attached to the amino acid. The DNP amino acids have a distinctive yellow color, and hence these combinations can be isolated and identified by chromatography.



It was shown that the molecule consists of two different polypeptide chains. By the DPN method it was found that the more acidic chain A has glycine as the end amino acid, while the more basic chain B has phenylalanine in the corresponding position. The long peptide chains were then split into smaller ones, usually di- or tripeptides, and this was done in various ways, so that the breaks in the chains occurred at different places, and consequently the peptide sequences "overlapped." By repeating the DNP technique, and by other means, the amino acid sequence could be elucidated. The two chains are believed to be held together by —S—S— bridges, but the exact points of union are not known, although they are undoubtedly where some of the cysteine radicals are located.

The two chains are shown below, together with a key to explain the abbreviations.

## CHAIN A

Gly.Ileu.Val.Glu.Glu.Cy.Cy.Ala.Ser.Val.Cy.Ser.Leu.Tyr.Glu.Leu.Glu.Asp.Tyr.Cy.Asp.  
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

## CHAIN B

Phe.Val.Asp.Glu.His.Leu.Cy.Gly.Ser.His.Leu.Val.Glu.Ala.Leu.  
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Tyr.Leu.Val.Cy.Gly.Glu.Arg.Gly.Phe.Phe.Tyr.Thr.Pro.Lys.Ala.  
 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30



<i>Amino Acid</i>	<i>Abbreviation</i>	<i>Amino Acid</i>	<i>Abbreviation</i>
Cysteine	Cy.	Valine	Val.
Aspartic Acid	Asp.	Leucine	Leu.
Glutamic Acid	Glu.	Isoleucine	Ileu.
Serine	Ser.	Phenylalanine	Phe.
Glycine	Gly.	Proline	Pro.
Threonine	Thr.	Histidine	His.
Alanine	Ala.	Lysine	Lys.
Tyrosine	Tyr.	Arginine	Arg.

**THE MOLECULAR WEIGHTS OF PROTEINS.**—The proteins all have extremely high molecular weights, but the usual methods of organic chemistry are not suitable for their determination. Physical methods have been evolved for this purpose. Because of their colloidal nature they can be pulled out of solution by the ultracentrifuge; i.e., centrifugation at enormous speeds. Precipitation in this way depends on the speed in relation to the weight, and suitable calculations permit the estimation of the molecular weight. The membrane method has also been used. That is, the size of the molecule is estimated by determining the size of the pores of a membrane through which it will pass. Another method is to measure the osmotic pressure of a protein solution and use the data thus obtained. The composition of the protein in terms of its amino acid content frequently gives very useful proportionate data. If the protein contains a heavy metal, its percentage determination is very indicative, since the protein must contain at least one atom to the molecule.

### Nutritional Importance of Proteins

Proteins are the basis of protoplasm, living tissue. At any rate, no living tissue is known that does not contain some protein. The proteins are nitrogenous compounds but they cannot be synthesized by the animal organism from atmospheric nitrogen or inorganic nitrogenous salts. The animal eats protein, either animal or vegetable, digests it to amino acids, absorbs them, and from them forms its own protein. The amino acids are the units, the building stones from which the complex structure is built. This being the case, the question of the importance of the various individual amino acids arises. Are they all equally valuable, or are some more important than others? A great deal of work has been done in this field and is continuing, with more particular application to human nutrition.

Investigators in this field include Osborne and Mendel, F. G. Hopkins, Sherman, and Rose. The type of the earlier experiments was as follows: Young animals (usually the standard white rat) were fed highly purified foods. The diet was calculated to contain all necessary nutritional factors, the protein alone being limited in kind or in amount. At first, single purified proteins were included as the sole source of protein. Some were found to be quite satisfactory for growth, others were found capable of maintaining the animal but at a stationary weight, and still others did not even permit the animal to remain at its initial weight. In Fig. 10 are shown growth curves illustrating these typical experiments with single purified proteins and in Fig. 12 are given pictures of the animals showing the kind of results obtained. It is evident that casein is a very good protein, gliadin is not so good, and zein is very poor, *when fed as the only protein*. A glance at Tables IX and X will show that casein contains little glycine but is nevertheless suitable for growth; that gliadin is moderately low in lysine and tryptophan; and that zein is very



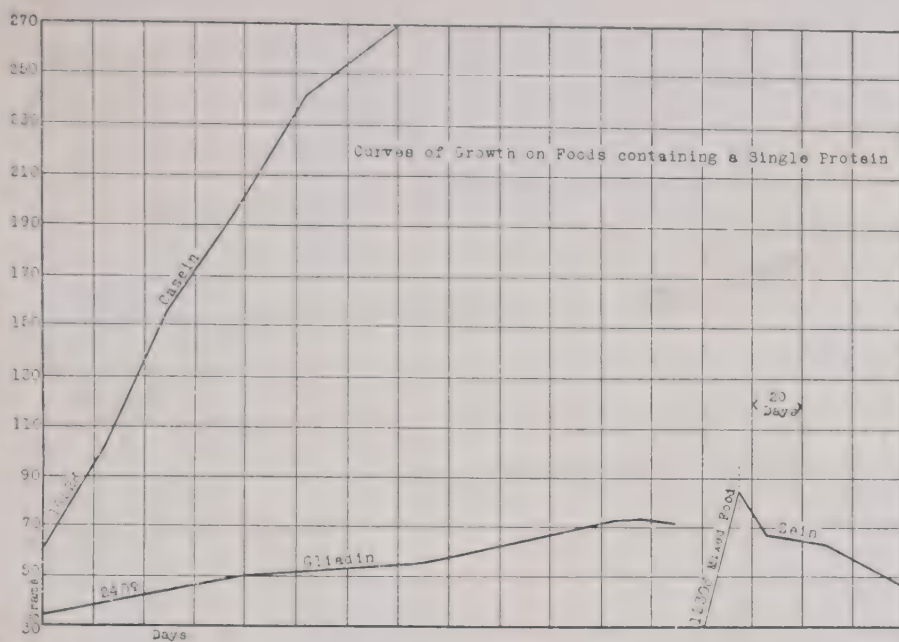


Fig. 10.—Showing typical curves of growth of rats on diets containing a single protein. On the casein food (devoid of glycine), satisfactory growth is obtained; on the gliadin food (deficient in lysine) little more than maintenance of body weight is possible; on the zein food (devoid of glycine, lysine, and tryptophan), even maintenance of body weight is impossible. (From Mendel, L. B.: J. A. M. A. 64: 1539, 1915.)

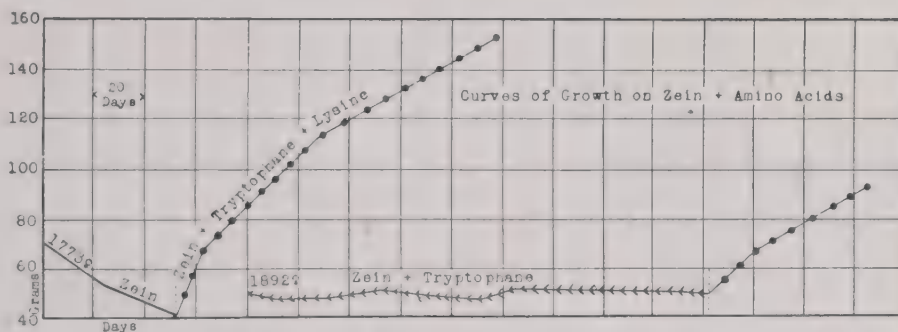


Fig. 11.—Showing the effect of the addition of tryptophan and lysine to zein, which is deficient in them. The addition of tryptophan permits maintenance without growth, while the further addition of lysine enables the animals to make considerable growth. (From Mendel, L. B.: J. A. M. A. 64: 1539, 1915.)

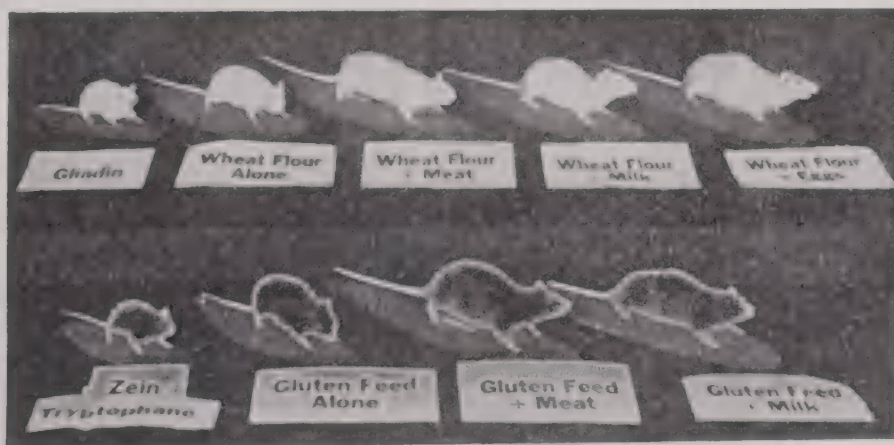


Fig. 12.—These rats were all of the same age and fed for the same length of time on diets containing the same proportion of protein. The variation in size is due to differences in the chemical constitution of the proteins eaten. (From experiments by Osborne and Mendel.) (From Mendel, L. B.: Nutrition: The Chemistry of Life, New Haven, 1923, Yale University Press.)

TABLE IX

APPROXIMATE PERCENTAGE COMPOSITION OF SELECTED PLANT PROTEINS\*

PROTEIN	CORN GLUTEN	WHEAT GLUTEN	SOYBEAN PROTEINS	YEAST PROTEINS	ZEIN	GLIADIN	EDESTIN
Nitrogen	(16.0)	(16.0)	(16.0)	(16.0)	16.1	17.7	18.7
Sulfur		1.1		0.8	0.52	1.24	0.88
Arginine	3.1	3.9	7.3	4.5	1.7	2.7	16.7
Histidine	2.1	2.2	2.9	3.0	1.3	2.3	2.9
Lysine	1.5	1.9	6.8	7.5	0	1.1	3.2
Tyrosine	6.3	3.8	4.0	3.6	5.3	3.2	4.3
Tryptophan	0.6	0.8	1.4	1.3	0.1	0.6	1.5
Phenylalanine	6.6	5.5	5.3	4.5	6.2	6.9	5.5
Cystine	1.5	1.9	1.9	1.1	0.8	2.6	1.2
Methionine	2.5	1.5	1.7	2.0	2.4	1.7	2.4
Threonine	4.0	2.5	3.9	5.5	3.5	2.1	3.9
Leucine	16.0	7.0	8.0	7.5	23.7	6.5	7.4
Isoleucine	5.1	4.2	6.0	6.0	7.3	5.4	4.6
Valine	5.7	4.1	5.3	5.8	3.5	2.7	5.7
Glutamic acid	24.5	27.0	18.4	14.7	26.9	45.7	20.7
Aspartic acid			3.7		6.6	1.3	12.0
Glycine	4.3	7.0	4.0	4.0	0.4	<0.5	5.1
Alanine		2.8	3.3		11.6	2.1	5.5
Proline		8.0	5.0		10.5	13.4	4.3
Serine		4.0	4.2		8.3	4.9	6.3
Sum	84	88	89	67	120†	105†	113†

\*Courtesy Dr. Richard J. Block.

†The explanation for this total of more than 100 per cent is that in hydrolysis water is added. Therefore, if the analyses were complete, each would total over 100 per cent. Of the substances analyzed, only zein, gliadin, and edestin are purified proteins; the others are mixtures.

TABLE X

APPROXIMATE PERCENTAGE COMPOSITION OF SELECTED ANIMAL PROTEINS\*

PROTEIN	GELA- TIN	ELAS- TIN	HORSE HEMO- GLO- BIN	EGG ALBU- MIN	IN- SULIN	PEP- SIN	WOOL	CASE- IN	$\beta$ -LACTO- GLOBU- LIN	BEEF MUS- CLE	SILK FIBRO- IN
Nitrogen	18.0	17.1	16.7	15.5	15.7	15.4	16.0	15.6	15.6	16.0	18.7
Sulfur	0.5	0.17	0.58	1.83	3.33	0.94		0.8	1.68	1.1	0.0
Arginine	8.6	0.9	3.7	5.9	3.1	1.0	10.1	4.1	2.9	7.7	1.1
Histidine	0.7	<0.1	8.7	2.6	4.9	0.9	1.0	3.1	1.7	3.3	0.4
Lysine	5.0	0.5	8.5	6.5	2.5	1.6	3.1	8.2	11.3	9.0	0.7
Tyrosine	1.0	1.6	3.0	3.7	13.0	8.5	5.5	6.3	3.7	4.0	12.8
Tryptophan	0	0	1.7	1.2	0.3	2.4	1.5	1.2	1.9	1.4	0.0
Phenylalanine	2.4	5.0	7.7	7.7	8.1	6.4	4.0	5.0	4.8	5.0	3.4
Cystine	0.1	0.6	1.0	2.8	12.5	2.1	13.6	0.35	3.5	1.2	0.0
Methionine	0.9	0.3	1.0	5.3	0.3	1.7	0.7	3.4	3.2	3.2	0.0
Threonine	2.2	1.3	4.4	4.0	2.1	9.6	6.5	4.9	5.5	5.0	1.6
Serine	3.4	0.8	5.8	8.2	5.2	12.2	7.4	7.7	4.4	6.0	16.2
Leucine	3.2	8.7	15.2	9.9	13.2	10.4	8.6	9.2	15.2	8.0	0.9
Isoleucine	2.1	4.0	0.2	7.0	2.8	10.8	4.3	6.1	7.3	6.0	1.1
Valine	2.7	17.4	9.0	8.8	7.8	7.1	5.4	7.2	6.2	5.5	3.6
Glutamic acid	10.8	2.1	8.2	16.5	18.6	11.9	14.0	23.3	21.5	17.0	2.2
Aspartic acid	7.5	0.6	10.6	9.3	6.8	16.0	7.4	7.1	11.4	10.5	2.6
Glycine	29.3	29.9	5.6	3.1	4.3	6.4	6.8	2.7	1.5	5.0	43.6
Alanine	10.0	18.9	7.4	6.7	4.5		4.0	3.0	7.1	7.4	29.7
Proline	16.5	17.0	8.5	3.8	2.5	5.0	8.0	11.3	5.0	6.0	0.7
Hydroxy-proline	14.0	2.0	0	0.0	0.0		6.7	0.0	0.0	1.0	
Sum	120†	112†	110†	113†	113†	114†	118†	114†	118†	112†	120†

\*Courtesy Dr. Richard J. Block.

†See footnote to Table IX for explanation of totals over 100 per cent.

low in glycine, lysine, and tryptophan. This would indicate that the ingestion of glycine is not essential for growth, whereas the ingestion of lysine and tryptophan is. The experiment shown bears this out. To the imperfect protein, zein, are added the two amino acids, tryptophan and lysine. Both are needed to supplement the deficiency and permit growth of the animal.

Other methods of experimentation have been devised. Protein hydrolysates have been prepared and, after removing a specific amino acid, they have been fed to the experimental animals to determine whether removal of this acid affects their nutritive well-being. More recently, mixtures of *pure amino acids* have been fed and, although such mixtures are very difficult to prepare and are very expensive, the results obtained have been quite valuable. From all of these investigations has come the realization that certain amino acids are essential or indispensable; that is, they must occur in the food if the young animal is to grow. They cannot be synthesized by the animal organism. At present the amino acids are classified, from this standpoint, as shown in Table XI.

TABLE XI  
CLASSIFICATION OF THE AMINO ACIDS WITH RESPECT TO THEIR INDISPENSABILITY FOR GROWTH\*

INDISPENSABLE	DISPENSABLE
Lysine	Glycine
Tryptophan	Alanine
Histidine	Serine
Phenylalanine	Aspartic acid
Leucine	Glutamic acid
Isoleucine	Proline
Threonine	Hydroxyproline
Methionine	Citrulline
Valine	Tyrosine
Arginine†	Cystine

\*After Rose, W. C.: Science 86: 297, 1937.

†Arginine occupies an intermediate position, since it can be synthesized by the animal but not rapidly enough to permit normal growth.

In addition to the indispensability of certain amino acids for growth, it is more than likely that some of them are essential to the maintenance of health in adults as well as in the young. A lack of tryptophan, histidine, or phenylalanine is one cause of cataract formation in rats (Bowles and others), and other effects observed following tryptophan deficiency have been defects in teeth, alopecia, hypoproteinemia, hypochromic anemia, atrophy of the testes, and other effects upon the reproductive organs.

Experiments have been cautiously extended to the human race to ascertain whether the same indispensability of amino acids applies. The chief criterion has been the determination of nitrogen balance; that is, a comparison of the amount of nitrogen excreted with that ingested in the food (see Chapter 15). Normally, the amounts are about the same. If, following the feeding of a food mixture, there is a net loss of nitrogen, the assumption is that the food is not adequate to maintain the tissues of the body. As a consequence, tissue protein is broken down and more nitrogen is lost than is taken in. Feeding a casein hydrolysate, from which individual amino acids were removed, to human



subjects was the method used in Holt's laboratory. Tryptophan, lysine, and methionine were found to be necessary for nitrogen equilibrium in man. Rose has in recent years done much work on this problem, using pure amino acids in the ration which he administered to the human subjects and following their nitrogen equilibrium over a considerable period of time (Fig. 13). From his experiments he concludes that only eight amino acids are essential to young healthy, adult males: lysine, tryptophan, phenylalanine, leucine, isoleucine, threonine, methionine, and valine. In these experiments neither histidine nor arginine was found to be indispensable; i.e., man can synthesize them if they are not present in the diet. These investigators have also determined the minimum daily requirement of the eight indispensable amino acids (See Table XII). It must be remembered that this was determined on a group of healthy young adult males and consequently cannot be taken as the standard human requirement under all conditions.

TABLE XII

MINIMUM AND RECOMMENDED INTAKES OF INDISPENSABLE AMINO ACIDS FOR YOUNG HEALTHY MEN WHEN DIET FURNISHES SUFFICIENT NITROGEN FOR SYNTHESIS OF NONESSENTIALS (STRICTLY TENTATIVE VALUES)\*

AMINO ACID	MINIMUM DAILY REQUIREMENT (GM.)	RECOMMENDED DAILY INTAKE (GM.)	SUBJECTS TESTED
L-Tryptophan	0.25	0.5	31
L-Phenylalanine	1.10	2.2	22
L-Lysine	0.80	1.6	27
L-Threonine	0.50	1.0	19
L-Valine	0.80	1.6	23
L-Methionine	1.10	2.2	13
L-Leucine	1.10	2.2	8
L-Isoleucine	0.70	1.4	8

\*From Rose, W. C.: Amino Acid Requirements of Man, Fed. Proc. 8: 546, 1949.

Since it is evident that no one eats purified proteins, or mixtures of amino acids, we must ask ourselves how this affects us from a practical standpoint. Are the proteins of any foodstuff deficient in one or more of the essential amino acids? Whole corn is low in tryptophan and lysine, wheat gluten is low in tryptophan, lysine, and threonine, and gelatin is deficient in tryptophan, leucine, isoleucine, threonine, methionine, and valine. Except for gelatin, hemoglobin, and keratin, the animal proteins are, in general, well balanced in their amino acid distribution. Yeast, corn germ, wheat germ, and soybeans yield approximately the same proportions of amino acids as do the animal proteins (Block and Bolling). The analysis of many foodstuffs for their amino acid content is not yet available. However, it is apparent that a mixed diet, containing some animal protein—milk, meat, fish, eggs—will insure the ingestion of an adequate mixture of the essential amino acids.

**Assay of Amino Acids.**—The analysis of pure proteins, of protein-hydrolysates, or of protein-containing foods for their content of the various amino acids is frequently required. However, the chemical methods available are, in many cases, difficult and time consuming. Other methods have accordingly



been devised, the most important of which are the microbiological and the chromatographic procedures.

**MICROBIOLOGICAL ASSAY.**—Some of the amino acids are as essential to the life of microorganisms as they are to animals. This fact has been utilized in the various "microbiological" assay methods. *Lactobacillus casei*, for instance, requires the presence of at least sixteen different amino acids in its medium if adequate growth is to occur. There is a direct relationship between the amount of each of these amino acids present in the medium (below its optimal value) and the amount of growth of the organism. Consequently if a medium is made up which is lacking in one of these amino acids, and the substance to be analyzed is added, the amount of growth will depend upon the amount of that particular amino acid in the "unknown." The quantitative estimation of growth in the case of *Lactobacillus casei* is made by determining the amount of lactic acid produced by this bacillus, either by titration or by measuring the shift of pH.

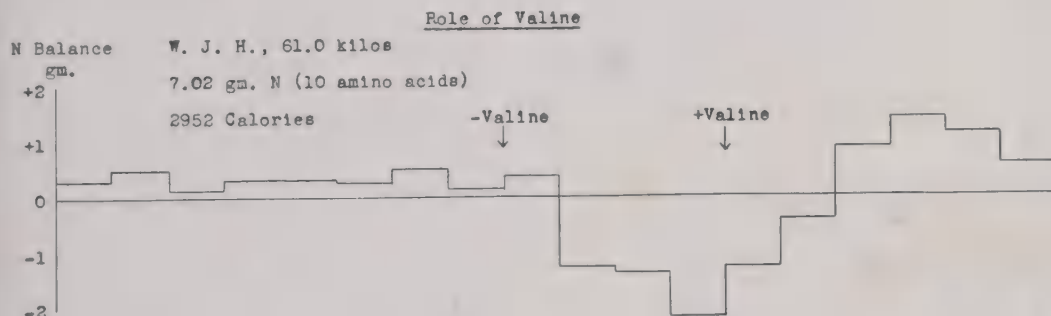


Fig. 13.—Determination of the indispensability of an amino acid for man. The initial diet contained 6.7 grams of nitrogen in the form of a mixture of the ten amino acids previously found to be essential for the growth of animals. The remaining nitrogen came from other components of the ration. The total nitrogen content of the diet was kept constant throughout. Valine was removed from the food at the first arrow and was returned at the second arrow. The horizontal units represent single days. (Courtesy Prof. W. C. Rose.)

Microbiological assays may also be done (1) by observing and measuring the production of gas by yeast, (2) by determining the degree of turbidity resulting from multiplication of the microorganism, and (3) in the case of molds, by actually weighing the mycelia formed. In all cases curves must first be prepared from determinations of known amounts of the amino acid, using the selected organism under carefully standardized conditions. Naturally the organisms will vary in their behavior, since all amino acids are not indispensable to the same extent in all microorganisms. Mutants of *Neurospora*, the red bread mold, produced by the action of x-ray, have been found to be very valuable in this type of work. These are distinct strains which are unable to manufacture certain nutrients; for example, definite amino acids or vitamins. For example, a given mutant, unable to synthesize methionine, will not grow in a medium lacking this amino acid. Therefore, if one wishes to analyze an hydrolysis mixture for methionine, this mixture would be added to the methionine-free medium in definite proportion and the amount of growth of the mold would indicate the methionine content of the unknown.

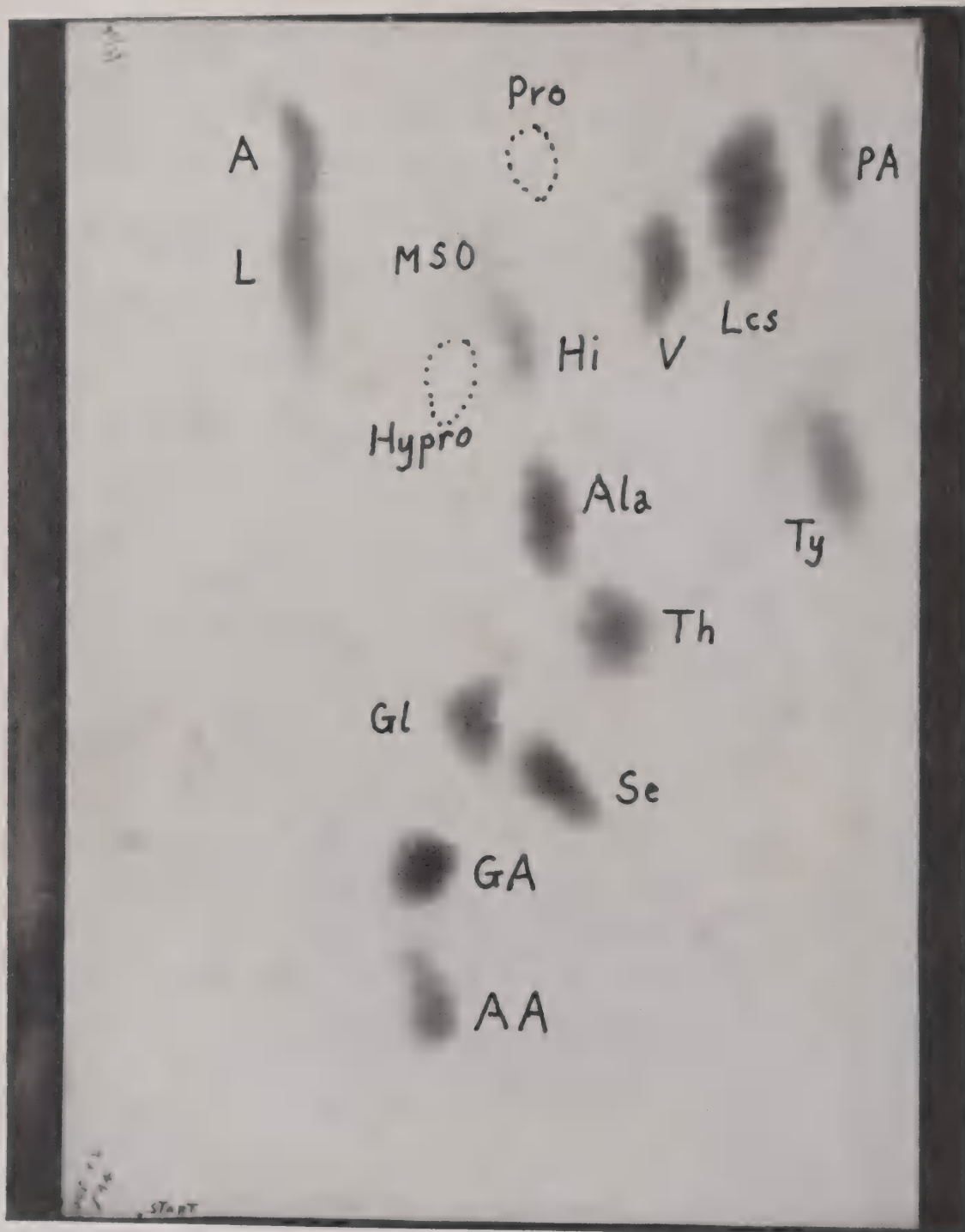
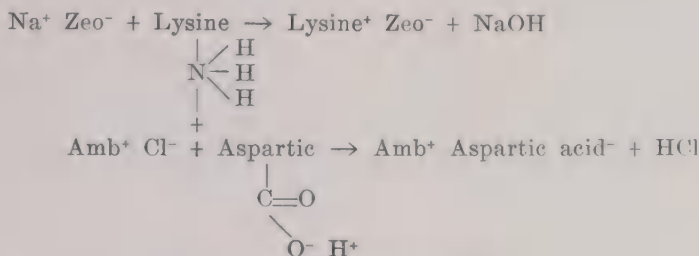


Fig. 14.—A two-dimensional paper chromatogram. A measured drop of the amino acid mixture was placed on the dot at the lower left-hand corner of the 19 by 22 inch sheet of filter paper and was dried. The lower edge of the paper was then dipped in a trough of aqueous phenol and the paper was suspended over glass rods. The solvent climbed by capillary action up the paper, carrying with it the amino acids. These were deposited along a vertical line. The paper was then turned at right angles so that the vertical left-hand edge became the bottom. This edge was then dipped into the second solvent, lutidine-alcohol-water, and the process was repeated, thus separating the spots of amino acids still more. At the completion of the run the paper was dried and sprayed with ninhydrin to develop the color. Violet to purple colors were produced except in the case of proline and hydroxyproline, which gave yellowish hues. The amino acid spots are identified as follows: A, arginine; L, lysine; MSO, methionine sulfoxide; Hi, histidine; V, valine; Lcs, leucine and isoleucine; PA, phenylalanine; Ty, tyrosine; Ala, alanine; Th, threonine; Gl, glycine; Se, serine; GA, glutamic acid; AA, aspartic acid; Pro and Hypro (yellow), proline and hydroxyproline. (Courtesy Dr. Richard J. Block.)

**ION EXCHANGE AND CHROMATOGRAPHIC ANALYSIS OF AMINO ACIDS.**—Besides chemical methods of separation and estimation of amino acids and the microbiological assay techniques described above, amino acids may be separated from each other in hydrolysis mixtures by other procedures. Ion exchange materials will selectively adsorb basic or acidic amino acids as the case may be. Thus arginine and lysine may be exchanged for  $\text{Na}^+$  on the synthetic cation exchange zeolite "permutit" (see page 81) (Whitehorn), and dicarboxylic amino acids are adsorbed on an anion exchange resin (Amberlite I R - 4) (Cannan). The amino acids may then be eluted by a suitable reagent.



Chromatography (see page 42) may be used to separate the amino acids and in some instances depends upon the same forces as operate in the case of the ion exchangers. Paper chromatography is a recent and very interesting development. Fig. 14 shows a paper chromatogram. A mixture of amino acids dissolved in a minute amount of fluid was placed in the lower left corner of the sheet of filter paper and dried. The sheet was then dipped into a solvent, aqueous phenol, and as this was drawn up into the paper, the amino acids were drawn with it *but at different rates*. Thus they were separated from each other but not completely. To accomplish complete separation, a second solvent, lutidine-alcohol-water, was used, and the sheet was turned at right angles. This two-dimensional chromatogram was then sprayed with ninhydrin so that the spots would become visible as purplish areas. (See Fig. 14.) The identity of the amino acid responsible for each spot is ascertained by running a control of pure amino acid solution. Besides qualitatively determining the amino acids, a fairly accurate quantitative measurement is possible by determining the maximum density of the color of the spot.

**Estimation of Proteins.**—For practical purposes, the amount of protein in a food is determined by analyzing for nitrogen. Proteins in general contain about 16 per cent nitrogen. Therefore,  $\text{N} \times 6.25 = \text{percentage of protein}$ . This is not entirely accurate since many proteins vary considerably from this 16 per cent figure; egg albumin, for instance, has 15.5 per cent and edestin 18.6 per cent. Furthermore, some of the nitrogen present may not be in the form of protein. Nitrogenous extractives, such as creatine and purines, are examples. The nitrogen is estimated by the Kjeldahl method.

**KJELDAHL METHOD.**—The weighed or measured nitrogenous material (food in this instance) is placed in a special Pyrex flask and to it is added concentrated sulfuric acid. This is then heated directly over a flame until the "moist combustion" is complete. Usually a



catalyst is added to hasten the reaction. All nitrogen compounds, except nitrites and nitrates, are converted by this reaction to  $(\text{NH}_4)_2\text{SO}_4$ . After cooling and suitable dilution, the mixture is made strongly alkaline and the  $\text{NH}_3$  distilled over into a known quantity of standard acid. Titration with standard alkali permits estimation of the amount of acid neutralized by the  $\text{NH}_3$ . Micromethods are also available, and the  $\text{NH}_3$  may be determined colorimetrically.

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## Chapter 6

### TISSUES

#### THE CELL

The typical animal cell consists of a cell membrane which surrounds and encloses the cytoplasm. In the cytoplasm is found the nucleus and other structures, some microscopically visible, such as mitochondria, and others of sub-microscopic size. The identification of characteristic substances in morphological structures is now being studied by histochemical methods. Histochemistry is a comparatively new field, but it is making rapid strides as new methods are being developed and applied. Thus far, these fall into four general groups. In the first, the substance is stained *in situ* or the products of enzyme action are so stained and the location of the substance or enzyme is made visible under the microscope. If the substance to be detected is highly diffusible, the tissue is frozen, serial sections are made, and the sections are dried in the frozen state under reduced pressure. In the second general procedure the sections are not stained but absorption of specific radiation is used to localize and even to determine quantitatively the absorbing substances. For example, nucleic acids absorb ultraviolet radiation of 260  $m\mu$  wave length and can be located and measured by absorption of this radiation. The third general procedure is that developed particularly by Linderström-Lang and Holter. Serial sections of a tissue are made and alternate sections are studied histologically and microchemically. For example, a number of pieces of gastric mucosa are punched out and of each one serial sections are made. The cells of every other section are counted and tabulated; i.e., the number of chief, parietal, and other cells is studied statistically. At the same time, microchemical analyses for pepsin, for example, are made on the other sections. By such a combination of methods the location of the pepsin in the chief cells has been demonstrated (see Chapter 10). Still another method is the separation of the various sorts of particles by ultracentrifugation. The cell membranes are first mechanically ruptured and the cell contents are suspended in a liquid medium and then subjected to high-speed centrifugation. In this way the different constituent parts are separated. Their identity can be established by microscopy and then the chemical and physical characteristics of each studied.

Fig. 15 shows a diagram of a typical cell, in this case a liver cell, with indications of various cellular structures.

**The Cell Membrane.**—The chemical nature of the typical cell membrane is not known, but from histological evidence and physiological behavior some hypotheses have been formulated. Thus, certain dyes act as “vital stains”; that is, they penetrate the cell walls of living cells and color the contents without injury. Among these stains are neutral red, methylene blue, and others

soluble in cholesterol, phospholipids, and the cerebroside, whereas dyes which are, in general, insoluble in the lipids do not penetrate the cell. This leads one to believe that lipids are present in the cell membrane as well as in the cytoplasm. The membranes are permeable to water, to some, but not all, ions, to food solutes, and to outgoing waste products. These and other considerations have led to the hypothesis that the cell membrane of a typical cell may be a

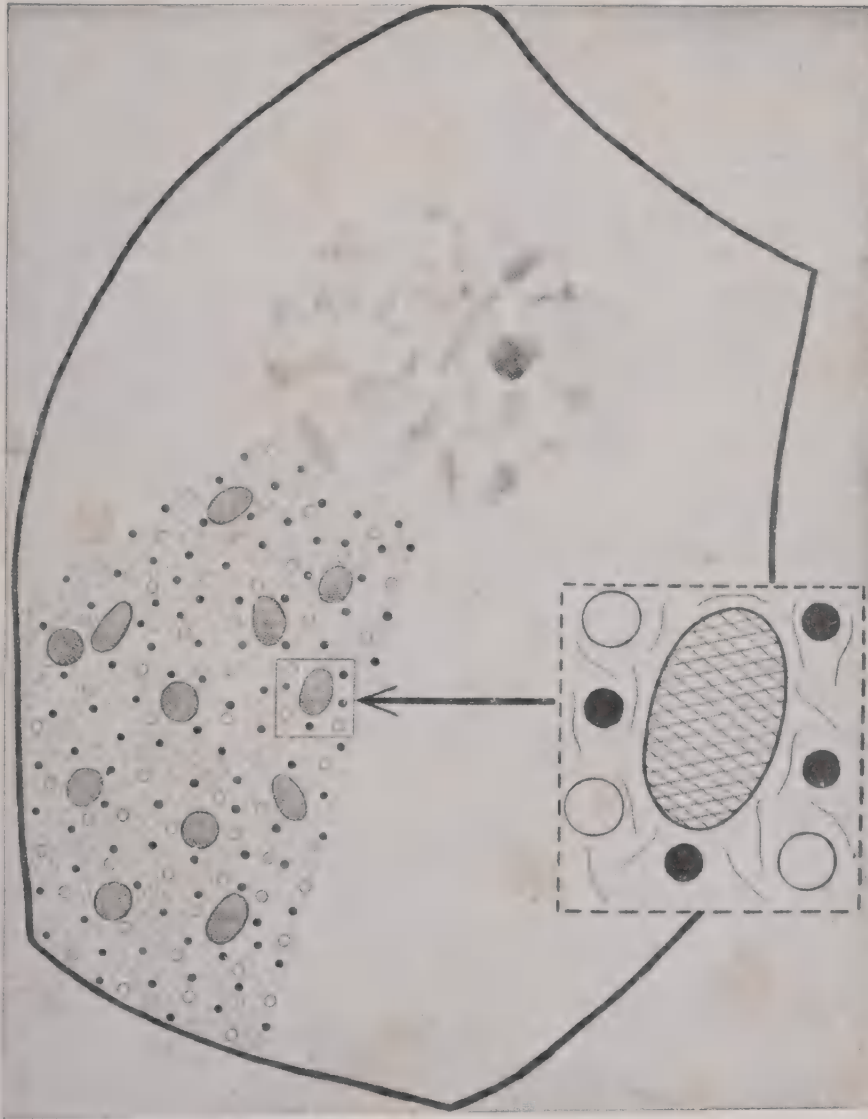


Fig. 15.—Schematic view of liver cell showing nucleus, cytoplasm, and cell membrane. Mitochondria, large cross-hatched bodies; submicroscopic lipoprotein complex, small solid spheres; particulate glycogen, hollow spheres; asymmetric micelles probably responsible for sol-gel changes, long narrow threads. (From Lazaraw, Arnold: *Biological Symposia* 10: 9, 1943, The Ronald Press Co.)

mosaic. Some parts of the patterns may be sievelike to permit particles of the solutes to pass through according to their size. Other sections may be solvent-like surfaces which dissolve certain solutes in them, permitting them to pass in or out as the case may be. Still other parts may consist of films of monomolecular dimension.

**The Nucleus.**—The nucleus contains a comparatively large amount of a particular nucleoprotein, located in the chromosomes. The nucleoprotein present yields, on hydrolysis, desoxyribose nucleic acid and a protein (see Chapter 15). Since the other type of nucleoprotein, yielding ribose nucleic acid, is found in cytoplasm, the terms nucleoprotein and nucleic acid are really not appropriate. The protein constituents of the nucleoproteins are histones and protamins. The amount of desoxyribose nucleic acid present is a constant for the different cells of the same organism and is twice the amount found in the sperm cell of that organism. The protein with which the nucleic acid is combined is not constant quantitatively, although just as important from a functional standpoint. (Mirsky.) The inorganic elements of the nucleus include calcium and magnesium with little potassium, except perhaps in the nucleolus. Most of the potassium is in the cytoplasm.

**The Mitochondria.**—The mitochondria are present in practically all living cells. They are visible in the living cell and may be stained with Janus green supravitaly; that is, the dye is applied to the living cell, which is killed by this action. They were separated by Bensley and Hoerr and have been analyzed. They are yellow in color and contain cholesterol, proteins, including nucleoprotein, and large amounts of phospholipid and fat. The color is due to the presence of a flavoprotein. The mitochondria are the site of many enzymes, perhaps several hundred.

**Submicroscopic Structures.**—Several different submicroscopic structures have been described. These can be observed with the use of the ultramicroscope and they, too, have been separated by special methods. One of these, found in the live cell, is a lipoprotein complex, containing fats, proteins, and nucleoproteins. It is cherry red when obtained in sufficient amount for observation. It has a higher content of lipids, nucleoprotein, flavoprotein, and water than mitochondria. The color, again, is ascribed to the presence of a flavoprotein. Another type is "particulate glycogen." This consists of glycogen with a small amount of protein and a considerable amount of water. A threadlike micelle is a third submicroscopic component. Some authorities believe that a micellar structure of protoplasm accounts for a number of its peculiar properties. Local changes in micellar forces, which undoubtedly occur in protoplasm, could account for changes from the sol to the gel form and vice versa, and these appear to be related to amoeboid movement, cell division, and other cellular activities. Other demonstrable organized material in the cytoplasm are the Nissl bodies in nerve cells and the Golgi apparatus in nervous and other tissues. Neither can be seen in living unstained tissue, but there seems to be no doubt that they represent structures present in living cytoplasm. The Nissl bodies consist chiefly of nucleoproteins, probably similar to yeast nucleic acid (Caspersson).

Between the particulate and micellar constituents, there is a continuous aqueous phase which is not visible under the microscope. This contains soluble proteins; organic substances ready for utilization, such as glucose; products of cellular activity, such as creatinine; and electrolytes. A large proportion of the cellular proteins is probably in this form; i.e., not a part of the structural



or particulate components of the cell. Some of the enzymes present in the cell appear to be situated within or in close association with the mitochondria and the submicroscopic lipoprotein complex. Whether all of them are so located is not yet known, but such a position would permit the various functions related to sol-gel changes to occur without interfering with enzymatic reactions.

**Mineral Constituents of Tissues.**—The location of the mineral constituents of cells and intercellular substance has likewise been subjected to histochemical study. This is done by microincineration. The tissues are fixed in an ash-free fluid, imbedded in paraffin, and incinerated in a special device. The mineral residue is examined by reflected light in the dark field. The color and crystalline structure, in most cases, are the clues to qualitative recognition. By this method, as well as by the use of the electron microscope, it has been found that the distribution of minerals within the cell is not uniform. Certain minerals are localized in specific regions. For example, the calcium and magnesium are concentrated in the nucleus, the cell membrane, the anisotropic discs of striated muscle, the zymogen granules of the pancreas, and in a few other locations. Other salts are present in other parts of the cell.

When tissues are analyzed by the usual biochemical methods, as distinguished from histochemical methods, the morphological structures such as mitochondria, etc., are disintegrated and the typical substances described in the preceding chapters are found. It is by such methods that most of our information up to the present has been obtained, and much of this knowledge is being used by the younger science of histochemistry. As time goes on, the two types of study will undoubtedly merge, or they will become more and more closely correlated.

## GENERAL COMPOSITION OF TISSUES

All cells—at any rate, all mammalian cells—contain the following primary or essential constituents: water, proteins, lipids, carbohydrates, inorganic salts, and enzymes. The proteins always present include albumins, globulins, and nucleoproteins. Invariably, phospholipids and cholesterol are found. It is probable that small amounts of fats occur in all cells. Either glycogen or monosaccharide is available to every cell. In addition to these constituents, common to all cells, there are secondary or special constituents characteristic of the particular tissue in which the cell is found. In some cases the special constituent is simply an extraordinary amount of one of the essential constituents. For example, a cell of adipose tissue is characterized by a comparatively tremendous amount of lipid. Other examples of these special constituents are the keratin of epidermal tissues, the glycogen of liver cells, the hormones of the endocrine glands, and hemoglobin of the red blood cells. Finally, there is always a greater or smaller amount of intercellular material which often overshadows the cells themselves in importance. The peculiar characteristics of specialized tissues are due to the chemical or physical nature of some one or more of their secondary constituents or to the intercellular substances.

The proteins of the various tissues differ from each other in their amino acid content. This is not as great, for certain tissues, as one would expect,

however. The proportions of eighteen amino acids in human liver, heart, and muscle tissue were found to be remarkably similar, but those of the skin were quite different (Bocobo).

### Epidermal Tissues

The epidermis consists of several layers, the lowest layer of which is the most active physiologically and therefore contains the most water. This is the *stratum germinativum*. As the cells lose water and are displaced by new ones, they are moved toward the surface. The next layer, the *stratum granulosum*, is made up of cells containing granules which are deeply stainable by basic dyes. These granules are composed of an albuminoid called "keratohyaline." This is believed to be the precursor of "eleidin," a semifluid substance occurring in the next highest layer, the *stratum lucidum*. The cells composing the stratum lucidum are shiny and refractile and do not stain with basic dyes. Evidently some marked chemical change occurs in the transition of keratohyaline to eleidin. Another change occurs when eleidin is transformed into keratin, the characteristic constituent of the uppermost layer, the *stratum corneum*. This layer is not as refractile as the lower one, the cells are tightly packed, and the nuclei are gone.

Keratin is an albuminoid. It is probable that more than one keratin exists in human skin. The chief characteristic of insolubility in water and in all neutral reagents makes it an ideal protective covering for the body.

Other epidermal structures besides the skin, such as nails and hair, also consist largely of keratin. Keratins are soluble in strong acids on the application of heat and in strong alkalies, but undoubtedly this is accompanied by decomposition. They are also dissolved by sulfides of the alkalies and alkali earths. They are characterized chemically by their high content of cystine, lysine, and arginine. Feeding cystine to animals on a low protein diet promotes the growth of hair. The keratin molecule probably consists of polypeptide chains closely packed together and joined to one another by the  $-S-S-$  bonds of the cystine units. That is, half of each such cystine is situated in one chain and half in the chain next to it, and the disulfide linkage acts as a bridge to unite the two. This firm and close union is believed to account for the relative insolubility and indigestibility of these proteins. As previously stated, the keratins can be digested if pulverized very finely, thus mechanically breaking these disulfide bonds.

Skin also contains a small amount of lipids. In the subcutaneous tissues there is a considerable quantity of lipids, and it is interesting to note that about one-fifth of this lipid material consists of sterols. It is one of these sterols which on irradiation by ultraviolet light is changed to vitamin D. (See page 264.) Fat, fatty acids, and phospholipids are also present. Lanolin, or wool fat, which finds some use in medicine, consists of esters of palmitic, stearic, and oleic acids with cholesterol and other sterols. Human sebum contains a considerable amount of hydrocarbons, about one-third of which is squalene. Cholesterol and other lipids are also present, but there is very little protein. Cerumen, or ear wax, is composed of over 40 per cent proteins and about 13



per cent neutral fats, with smaller amounts of phospholipids, cholesterol, and other lipids. Carbohydrates in the skin include glucose and glycogen. It has been claimed that the skin may act as a temporary storage depot for glucose when this is present in the blood in large amounts. Inorganic radicals present in all human tissues are found in epidermal tissue. Toxic heavy metals, however absorbed, seem to find their way in part to the skin and are deposited there. In silver poisoning, argyrisms, the skin may become bluish in color and remain so for years.

The chief pigment of skin is *melanin*. This occurs in variable amounts as fine granules in the cells, and between the cells, of the stratum germinativum. Dark-skinned races have more than the white race and brunettes more than blondes, while albinos have none, probably as a result of the absence of an enzyme which produces it from tyrosine through 3,4-dihydroxyphenyl alanine. The latter substance is also called "dopa" for short, and the enzyme in the skin which converts it into melanin is tyrosinase. This conversion is aided by the "tanning" action of sunlight, but the mechanism of this action, as well as the structure of melanin itself, is unknown. Melanin is a dark brown substance, quite insoluble in all ordinary reagents except alkalies, although all melanins do not dissolve readily even in these reagents. The relationship of the melanins to tyrosine and adrenaline is discussed in Chapter 15.

### Connective Tissues

The connective tissues are composed of a relatively small number of living cells embedded in an intercellular material often called the matrix. It is this intercellular material which gives the different tissues their distinctive physical properties. Several albuminoids and conjugated proteins are present in these tissues in varying proportions. Collagen is one of the former. It is a tough fibrous material present in very large amount in the tendons. Like keratin, it is insoluble in the usual protein solvents; in fact, it is insoluble in everything which does not change it chemically. Tannic acid converts it into a very hard material which is resistant to putrefaction and is the basis of leather. Another most important chemical property is its transformation to gelatin. This may be accomplished by boiling it in water for a long period or in acid solution for a shorter time. While this is being done, the collagen swells up at first and then gradually goes into solution. Upon cooling and standing, the solution becomes a semisolid gelatinous mass. There is some question as to whether this change is a hydrolysis or an intramolecular rearrangement. It is surely a partial hydrolysis, because gelatin contains somewhat less tyrosine than collagen, but it may also be an intramolecular rearrangement. Collagen is slowly digested by pepsin and only slightly attacked by trypsin, but gelatin is rapidly digested. This indicates that the fibrous parts of meat are more easily digested after cooking. Because gelatin is so readily digested and can be prepared in very palatable and attractive forms, it is a common article of diet for invalids and convalescents. This is all right if not carried too far. Gelatin is by no means an adequate protein since it contains no tryptophan and insufficient amounts of certain other amino acids.

Elastin is similar to collagen in many of its properties. It is also tough and strong but is more elastic. It is found in the ligaments in large amounts and also in the walls of the blood vessels, in the lungs, and in other tissues where elasticity is needed. Like collagen, it is not confined to a few tissues, but fibers of both frequently are found in many locations. Collagens and elastins are the only proteins containing significant amounts of hydroxyproline, collagens having about 13.5 per cent and elastins about 2 per cent. (Neuman and Logan.) Elastin is also insoluble in all solvents which do not change its chemical nature. It is *not* converted into gelatin, as is collagen, but is slowly digested by proteolytic enzymes. It, too, is an inadequate protein, nutritionally. The white fibers of connective tissue differ from the yellow elastic fibers not only in color and in the properties mentioned, but also in the different staining reactions which are quite specific for each. The reticular fibers of reticular tissue contain "reticulin," an albuminoid which resembles collagen very closely. In the subcutaneous tissues are found collagen and reticulin.

Mucoids are present in all the connective tissues. They are glycoproteins which on hydrolysis yield a protein and a mucopolysaccharide, or acid polysaccharide, such as chondroitin sulfuric acid (or chondroitic acid), hyaluronic acid, or mucoitin sulfuric acid. Chondroitin sulfuric acid has, as its hydrolytic products, sulfuric acid, acetic acid, glucuronic acid, and galactosamine. It occurs in a highly polymerized state, somewhat like starch and glycogen. Hyaluronic acid (see page 102) yields acetic acid, glucuronic acid, and glucosamine as its hydrolytic products. Mucoitin sulfuric acid differs from chondroitin sulfuric acid only in having glucosamine in place of galactosamine. Mucoitin sulfuric acid occurs in the mucus secretion of epithelial cells, while chondroitin sulfuric acid occurs in connective tissue and its secretions, such as the synovial fluid in the joints. The mucoids are termed tendomucoid, osseomucoid, and chondromucoid, depending upon whether they are derived from tendon, bone, or cartilage, respectively. They may be obtained from the tissue by extraction with limewater, since the calcium salts are soluble. Subsequent acidification precipitates them from this solution. The composition of white fibrous and yellow elastic tissue of the ox is compared in Table XIII. It is to be noted that they resemble each other closely in all constituents except elastin and collagen, which are about reversed; i.e., white fibrous tissue is high in collagen and low in elastin and yellow elastic tissue is high in elastin and low in collagen. Both are extremely deficient in inorganic matter and coagulable protein and rather low in mucoids.

Cartilage is a hard homogeneous tissue, flexible when in thin layers. It resembles bone chemically but has far less mineral matter and far more water. Its organic basis is chiefly collagen, with another albuminoid, chondroalbuminoid, and some chondromucoid. Chondroalbuminoid resembles elastin in some respects, especially in its digestibility. Chondromucoid is very similar in composition to if not identical with tendomucoid. The collagen is the same as that found in the other connective tissues, and gelatin may be obtained very easily from it. Elastic fibers composed of elastin are present in elastic cartilage, tending to give added flexibility to this tissue. Articular cartilage is exceedingly



elastic. It will recover quickly and completely from repeated intermittent pressures. This property, which enables cartilage to absorb the shocks to which the body is subjected, is lost on drying but is regained when water is permitted to return. It is quite possible that vitamin D is in some way related to the development and health of cartilage in addition to being involved in its normal conversion into bone (see Chapter 12).

TABLE XIII

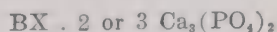
COMPARISON OF WHITE FIBROUS AND YELLOW ELASTIC TISSUE OF THE OX

	WHITE FIBROUS (BUERGER AND GIES) PER CENT	YELLOW ELASTIC (VANDERGRIFF AND GIES) PER CENT
Water	62.87	57.57
Inorganic matter	0.47	0.47
Lipids	1.04	1.12
Coagulable protein	0.22	0.62
Mucoid	1.28	0.53
Elastin	1.63	31.67
Collagen	31.59	7.23
Extractives, etc.	0.90	0.80

### Bone

True osseous tissue, as distinguished from bone marrow, contains only from 20 to 25 per cent water. This is much less than is found in most tissues and indicates that bone is not an active tissue physiologically. It is the structure which supports the body and protects various vital organs; consequently, it must have toughness, rigidity, and strength. It is not surprising then to find that the dry matter is made up of a large amount of mineral salts laid down in an organic matrix. About 60 per cent of the dry matter is inorganic and 40 per cent is organic. The organic basis of bone comprises a collagen, "osseine"; osseomucoid; and "osseoalbuminoid," an albuminoid closely resembling elastin. A proteinase and a peptidase have been found in the bones of young animals.

Analysis of the ash of bone reveals a great preponderance of calcium, a small amount of sodium, less magnesium, with still smaller quantities of potassium and other basic elements. The negative radicals are chiefly phosphate, with some carbonate and small amounts of chloride and fluoride. It is now generally considered that most of the inorganic matter of bone is in the form of complex salts (apatites) having the general formula:



in which B may be Ca or Mg, Na<sub>2</sub> or K<sub>2</sub>, and X may be CO<sub>3</sub>, (OH)<sub>2</sub>, F<sub>2</sub>, O, Cl<sub>2</sub>, or SO<sub>4</sub>. The salt present in largest amount is CaCO<sub>3</sub>·3Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and next is MgCO<sub>3</sub>·3Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. In lead and radium poisoning, these elements take the B position. However, no definite salt is present as an unchangeable combination. The bone salts continually change, one element or another moving in or out. They resemble lattices with the atoms entering and leaving the spaces in the crystal structure. Slight differences in pH and in the available concentrations of the necessary ions account largely for these variations.

The hardness and rigidity of bone are chiefly due to the inorganic salts, while the elasticity and toughness are attributable to the organic matter. This can be illustrated by two simple experiments. If a small piece of bone is incinerated, there will result a white ash having the form of the original fragment of bone. When this is ground in a mortar it will be seen to be brittle and can be ground to a fine powder, the hardness of which will be apparent. On the other hand, if a bone is subjected to the action of dilute HCl for a number of days, the inorganic salts will be removed and the material left will again be seen to have the exact form of the original bone. However, it will no longer be hard and rigid but tough and flexible. The combination of these inorganic and organic fractions results in a very strong tissue with high tensile strength. Since bone is laid down first as cartilage or membrane which becomes impregnated more and more with inorganic salts, it is easy to see why children's bones are less easily broken than adults'.

Citrate has been discovered as a constituent of human bone to the extent of about 1 per cent of the dry matter, which may constitute as much as 70 per cent of the citrate of the whole body. (Dickens.) When means are employed to increase the citrate excretion into the urine, this "extra citrate" does not come from the stores in bone. (Class.) Nevertheless the citrate content of bone is not constant and is therefore apparently available for some physiological need. Its exact function is not known. It may be a source of energy in bone or other tissue metabolism, but it probably has a special role in calcium metabolism by virtue of its power to bind calcium. This calcium citrate complex is soluble and diffusible but is un-ionized.

### Bone Formation

In the formation of the complex bone salt described, it is necessary that the concentration of the various ions involved exceed the saturation point at the site of deposition. Since calcium and phosphate make up the greater part of this salt, it is evident that these are the ions chiefly involved.

In the process of bone formation two enzymes appear to be involved: phosphorylase and phosphatase. The epiphyses of the bones of growing mammals have been shown to contain a phosphorylase. This enzyme catalyzes the interconversion of glycogen and glucose-1-phosphate. The phosphatase hydrolyzes this substrate as well as any other phosphoric acid esters which may be available. Thus the hexose-phosphates, glycerophosphates and nucleotides are potential sources of phosphate ions. The phosphatase which is found in high concentration wherever bone is being formed is probably produced by the osteoblasts, and the concentration of  $\text{PO}_4$  ions is raised locally near these cells as a result of enzyme action. Calcium is present in blood both in ionized and un-ionized form. The un-ionized calcium is partly diffusible and partly not diffusible. The nondiffusible calcium is largely that fraction combined with protein, while the calcium citrate complex forms most of the un-ionized diffusible part. Probably the chondroitin sulfuric acid of growing bone unites with calcium to provide a local surplus of available calcium. Normally the concentrations are such that the product of ionic calcium and phosphate is about 36 to 40

mg. per 100 ml. of plasma. Products above 40 are found when bone growth or healing is taking place, while products below 40 generally are seen in active rickets and in other conditions in which bone formation is not occurring properly. When the concentration of both calcium ions and phosphate ions is increased beyond the saturation point, the formation of colloidal calcium phosphate occurs. How this is changed to bone salt is a matter of dispute. One view is that it occurs in a series of steps until finally the apatite molecule is produced and additions or substitutions occur until a more stable and insoluble salt is achieved. This has been demonstrated by following the course of radioactive phosphorus,  $P^{32}$ . After administration, this is rapidly taken up by bone and built into the apatite structure. Enzyme conditions are favorable for the production of citric acid in calcifying areas of bone, and it is probable that citric acid is coprecipitated during deposition of bone salt. Citric acid is thus available for the re-resolution of calcium when that is brought about, and a high citric acid concentration could even reverse calcification and solubilize bone salt which has already been laid down. (Dixon and Perkins.)

The phosphatase is present in large amounts in the layer of osteoblasts on the surface of growing bone and in smaller amounts in adult bone but is absent from cartilage which is not undergoing ossification. The optimum pH at which phosphatase acts is an important factor. This is about pH 9.0. In this connection it should be noted that just prior to ossification chondroitic acid usually disappears from those areas in which osteoblastic activity is exerted. (Sylvén.) This tends to change the pH in an alkaline direction. However, phosphatase is present in certain tissues which do not ossify, e.g., the intestine, and it is absent sometimes when pathological calcification is taking place, as in the arteries. It is therefore evident that the phosphatase theory of bone formation (Robison's) does not tell the whole story. It is also significant that there is an accumulation of glycogen in the cells of epiphyseal cartilage prior to calcification. This is undoubtedly due to the presence of a phosphorylase, which thus provides not only organic phosphate, but also, perhaps, a supply of energy, which may be required in some enzymatic phase of calcification. (Gutman and Yü.)

It should be noted that the bone calcium is in equilibrium with the calcium of the blood. Consequently, the latter can be kept at a fairly constant concentration by a slight shift of calcium from the bones to the blood or vice versa. As mentioned previously, the bone salts are continually being absorbed and rebuilt. The salt formed is not of constant composition because the local conditions in the developing bone differ from time to time, as does also the chemical composition of blood. This process of decalcification and recalcification is under the control of various factors. Some of these are vitamins D, C, and A and the parathyroid and anterior pituitary gland secretions. It is self-evident that the amounts of calcium and phosphorus in the diet are important, as are various factors controlling their absorption and metabolism; e.g., amounts and proportions of dietary protein and fat, the acid-base balance, and the vitamins and hormones mentioned.



In rickets the amount of calcium phosphate in the bones is much below normal. This is usually the result of inadequate vitamin D which has an inhibiting effect upon the absorption and utilization of calcium and phosphorus. Since the concentration of minerals in the bones is low, they become less rigid and consequently bend, resulting in bowlegs or other deformities (see Chapter 12). Vitamin A deficiency retards the growth of bone, particularly the endochondral bone formation in rats. If the deficiency is established very early in life, skeletal growth is inhibited considerably before the effect upon total increase in weight can be observed.

Vitamin C also is essential to bone development. In scurvy there are lesions of the epiphyseal junction of growing bones. Subperiosteal hemorrhages are likely to occur both in growing and adult bone. Rarefaction of the alveolar bone leads to loosening of the teeth, dentine is resorbed, and the gums become spongy.

The parathyroid secretion has a regulatory effect upon blood calcium by removing calcium salts from the bones, thus tending to raise the calcium level of the blood. If this is carried to excess, the bones may be weakened. The anterior pituitary gland has a marked effect upon bone growth. In general, a hyperactivity causes an increased rate of growth as is seen in the overgrowth of the skull in acromegaly and in the huge bones in gigantism; a deficient activity is seen in the small bones of pituitary dwarfs. Thus both hormones and vitamins share in the complex metabolism of bones.

### Bone Marrow

Bone marrow is of two kinds: yellow and red. The yellow marrow is composed of connective tissue and very large amounts of fat. It has nothing to do with the function of forming red cells. That function belongs to the red marrow; but the yellow marrow may under some circumstances be converted into red marrow, which produces the red cells, some of the white cells, and perhaps the platelets (see Chapter 8). Red marrow is higher in protein but much lower in fat. Both types contain albumins, globulins, nucleoproteins, fibrinogen, polypeptides, phospholipids, cholesterol, and extractives.

### Teeth

The teeth resemble bone chemically to a certain extent. In Fig. 16 is shown a diagram of a typical tooth. Over the upper surface of the tooth one finds the enamel. This is the hardest substance in the body, a property of great value for the masticating and grinding action of the teeth. Only about 5 per cent of enamel is water. The remaining 95 per cent consists of an organic matrix of keratin impregnated with hydroxyapatite, a calcium phosphate with the formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . The greater part of the tooth is dentine, which is identical with bone from a chemical standpoint although different from it histologically. Dentine protein is largely collagen. (Block, Horwitt, and Bolling.) The inorganic basis is again an apatite, which *during growth* may be subject to continual changes in composition similar to those occurring in the bone salt. In fact, administration of labelled phos-



phorus is followed by rapid uptake of the tracer by developing teeth. Once the teeth are completely formed and calcified, this "continuing metabolism" is reduced to a minimum. Thus, the teeth are not drawn upon for calcium in time of need, as are the bones.

When the enamel breaks and the underlying dentine is exposed, *dental caries* develops. The cause of this formation of tooth cavities has been a matter of dispute for years and is still unsettled. It has been assumed that food particles lodge in the crevices after the enamel has cracked and decompose under the

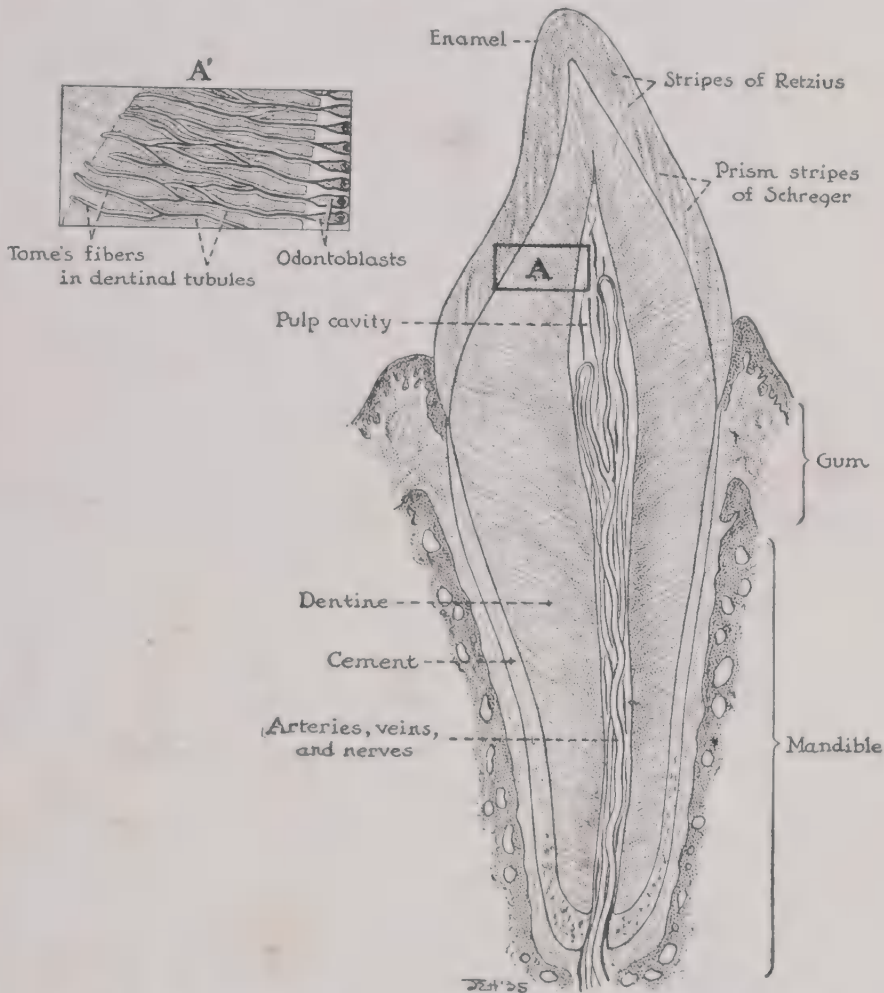


Fig. 16.—Longitudinal section of an incisor tooth. The part A is shown at high magnification in A'. (From McClendon, J. F., and Pettibone, C. J. V.: *Physiological Chemistry*, ed. 6, St. Louis, 1936, The C. V. Mosby Co.)

influence of microorganisms. This causes a local increase in acidity which results in further damage to the enamel. It is a fact that dental caries is less prevalent in regions where the drinking water is fairly high in fluorides. It is also known that fluoride, after ingestion, is deposited primarily in the enamel of the teeth and secondarily in the bones. It is claimed that if an optimum amount of fluorine is deposited, it prevents caries either by a chemical action or by a physical influence on the enamel. Too large an amount, however, causes the enamel to be mottled and brittle (see Chapter 18).

Vitamins A, C, and D are all necessary for proper tooth development and calcification. Lack of vitamins A and C affects the functional activities of the formative cells. A deficiency of vitamin A results in hypoplastic enamel, imperfectly calcified. A lack of vitamin C affects the calcification of dentine. Vitamin D not only aids in the absorption of calcium, but also promotes the deposition of calcium and phosphorus in teeth.

### Adipose Tissue

Two types of tissue fat are to be distinguished: protoplasmic fat (*élément constant*) and depot fat (*élément variable*). The former is an essential constituent of protoplasm and includes other lipids besides neutral fat. It is claimed that this fraction is not reduced in amount in starvation. The depot fat, on the other hand, is true adipose tissue and is largely a reserve food supply. A cell of adipose tissue is literally a droplet of fat contained in a thin membranous living cell. In order to get the fat out of such tissue, it must be heated, the cell membranes and supporting tissues ruptured, and the fat poured and strained off. When fat is "tried out" in this way, very little protein matter is left behind. Although the distribution of adipose tissue will vary in different individuals, in general a large part of it is in the subcutaneous tissue. Other locations are near the kidneys, in the omentum, and in most other tissues except the brain.

According to recent investigations, it appears that fat must be deposited in tissues before it can be utilized. Even when given in small amounts to a starving animal, it was not burned directly as so much fuel, but only after it had been incorporated into protoplasm.

In obesity there is an abnormal amount of fat laid down as adipose tissue. This is a result of a surplus of food or an insufficient amount of muscular work. However, there are a number of factors which enter into the problem (see Chapters 17 and 21). Excess obese tissue can be removed surgically and this is frequently done—usually for cosmetic reasons.

### Nervous Tissue

Nervous tissue makes up only about one-fortieth of the total weight of the body, yet the brain and nervous system dominate most of the functions of the body. This is done either directly by nerve impulses sent to the tissue or organ or indirectly by nervous control of the blood supply to them. We would expect, therefore, such a remarkable type of tissue to have a chemical make-up quite different from other tissues. This is true. Nervous tissue is characterized by the presence of a large proportion of lipids. Fat, however, is not among the lipids of nervous tissue. Like all other active tissues, there is a very large amount of water present—more in embryonic and in young nervous tissue and increasingly less with age. Nearly half of the dry matter of the human brain consists of proteins including albumin, globulin, collagen, nucleoprotein, and neurokeratin (see Table XIV). The gray matter is much richer in proteins than the white matter, as can be seen from Table XIV. Corpus callosum, which is composed entirely of white matter, contains 27 per cent proteins, and whole brain, 37 per

cent. The proteins of nervous tissue include an albumin, several globulins, a nucleoprotein, and neurokeratin. Neurokeratin is the material remaining after nervous tissue is subjected to digestion by gastric and pancreatic juices and then extracted with organic solvents, dilute acid, and alkali. This protein has the physical properties of keratins, to be sure, but contains the amino acids in very different proportions than are present in keratins obtained from true epidermal tissue. The amino acid content of this and of the combined brain proteins has been determined by Block. The relative amounts of cystine, tryptophan, histidine, tyrosine, lysine, and arginine are about the same in a number of different animals. There is a remarkable constancy in the ratio of lysine to arginine in all species except man and monkey. In the other species it is lysine:arginine = 100:103-105. In man and monkey it is 100:95-96. The percentage of nitrogen in brain proteins is rather low, namely, 13.4 per cent in human tissue. The nucleoprotein content is low but seems to contain a typical thymus nucleic acid. Glycogen also is low in nervous tissue; in other words, the brain has little reserve supply of carbohydrate. For its proper functioning it requires a normal concentration of glucose in the blood in addition to a small amount of hexosephosphate, which apparently comes into the tissue by diffusion. When the blood sugar becomes too low, as, for example, after an overdose of insulin, the brain is affected and symptoms of dizziness, mental confusion, weakness, delirium, and even convulsions may ensue. The insulin shock therapy for schizophrenia (dementia praecox) utilizes this mechanism with some degree of success. Small amounts of phosphocreatine, adenosine triphosphate, inositol, and other extractives\* are also present, as well as various inorganic salts, particularly alkaline phosphates.

TABLE XIV  
SOLIDS OF HUMAN BRAIN\*

	WHOLE BRAIN (CHILD) PER CENT	WHOLE BRAIN (ADULT) PER CENT	CORPUS CALLOSUM PER CENT
Proteins	46.6	37.1	27.1
Extractives	12.0	6.7	3.9
Ash	8.3	4.2	2.4
Phospholipids	24.2	27.3	31.0
Cerebrosides	6.9	13.6	18.0
Lipid sulfur	0.1	0.3	0.5
Cholesterol	1.8	10.9	17.1

\*From Koch, W.: *Ztschr. f. physiol. Chem.* 63: 432, 1909.

As can be seen from Table XIV the lipids are present in very large amounts in brain tissue. In adult brain they make up more than half of the total solids. The phosphatides are present most abundantly, next the glycolipids, then cholesterol, with the sulfolipids last. White matter contains more lipids than gray matter, in general. It should again be emphasized that no true fat is present in nervous tissue, and experimental work has shown that the metabolic "turn-

\*The term "extractives" is rather loosely applied to substances which may be extracted from tissues by boiling water. It usually excludes proteins and inorganic salts.

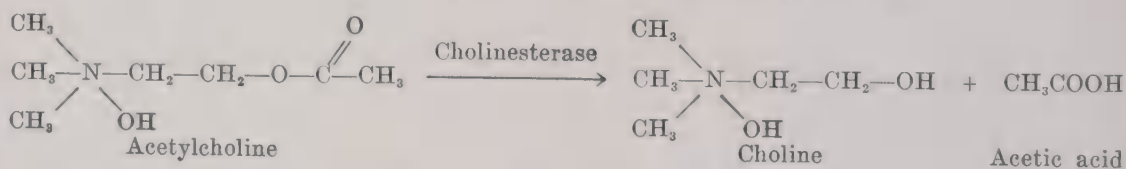


over'' of the fatty acids present is very slow in nervous tissue as compared with that in other organs.

Another marked chemical difference between gray and white matter is in the mineral content. The ash of the corpus callosum makes up 2.4 per cent of the solids, while it is 4.2 per cent of whole brain solids; that is, ash is higher in the gray matter than in the white.

*Acetylcholine and Sympathin.*—Although acetylcholine and sympathin are not present in any considerable amount in nervous tissue, their physiological role may be discussed at this time. The transmission of nervous impulses from one protoplasmic structure to another is today believed to be accomplished by means of chemical compounds. These compounds are produced or released upon stimulation of the nerve at the point where there is a junction with a second neuron (synapse) or at the receptor mechanism in the innervated tissue or organ. Unlike hormones, they effect their action at the site of production. It has been determined that acetylcholine is the substance liberated at all autonomic ganglia and at parasympathetic postganglionic endings. (There is evidence that it is also liberated at or in the myoneuron junction of striated muscle.) Otto Loewi was the first to discover the liberation of this compound and his work has since been extended by others, notably Dale and Nachmansohn. Cannon and associates have presented evidence in favor of the existence of sympathin, an analogous substance liberated at sympathetic postganglionic endings.

Acetylcholine has marked pharmacological properties when introduced intravenously into an animal. It slows the heart rate, dilates the arterioles, constricts the bronchi, and has many other effects. There is present in the blood stream, in the tissues, and especially in the axon and just beneath the cell surface of the ganglion cell an enzyme which is capable of hydrolyzing acetylcholine into choline and acetic acid compounds which are much less active than the parent substance. This enzyme has been named *cholinesterase*.



Sympathin is an epinephrine-like compound of undetermined structure having many properties which are similar to those of epinephrine, yet differing from it in others. It has no effect upon the dilator muscle of the iris, and the rise in blood pressure which it produces is not blocked after the administration of ergotoxine as is that of epinephrine.

The transmission of the nerve impulse along the axon and across the synapse has been claimed to be an electrical phenomenon by some investigators and a chemical one by others. It is not our purpose to enter into the discussion of the relative merits of either view, but the evidence seems to point to the impulse transmission as being both chemical and electrical in nature, with the chemical action being the primary event.



## Muscle Tissue

Muscle forms a very large proportion of the active tissue of the body. In normal adults it is fully two-fifths of the body weight, but about half of the metabolic, or chemical and physical, activity of the body takes place in our muscles even during rest. When the muscles are contracting, when they are doing work, fully three-fourths of the total metabolism can be assigned to them. The three types of muscle, voluntary, involuntary, and cardiac, differ somewhat in their chemistry, but they have the same general characteristics. In muscle we find:

Water

Proteins

Albumins, globulins, nucleoproteins, albuminoids

Lipids

Extractives

Nonnitrogenous: glycogen, glucose, inositol, hexosephosphates, lactates

Nitrogenous: creatine, creatine-phosphate, creatinine, inosinic acid, adenylic acid, adenosine triphosphate, glutathione, purines, pyrimidines, carnosine, anserine, choline, acetylcholine

Enzymes, hormones, vitamins

Inorganic salts

In the adult there is from 72 to 78 per cent of water, another example of the fact that water is essential for physiological activity. As in the case of nervous tissue, the water content of the muscle of the young and of the fetus is even higher. Here the similarity ends however. The solids of muscular tissue are largely protein in nature, whereas, it will be remembered, those of nervous tissue are largely lipids. The total lipids of muscle amount to only about 3 per cent and the glycogen is less than 1 per cent, but the protein content is about 19 or 20 per cent.

**Muscle Proteins.**—Striated muscle is composed of fibers made up of innumerable fibrils arranged parallel to each other and parallel to the axis of the fiber. These fibrils are about  $1\ \mu$  in diameter and are separated from each other by about half that distance. The fibrils are believed to consist of two proteins, actin and myosin, which form a complex called actomyosin, while the sarcoplasm which surrounds them is a mixture of globulin-like and albumin-like proteins. Around and among the muscle cells are found connective tissue fibers which ultimately become attached to the tendon. These consist of collagen, elastin, and some vascular substance and comprise the extracellular proteins, whereas the proteins of the fibrils and sarcoplasm are intracellular.

If fresh muscle is hashed and extracted with dilute KCl at pH 7-8, a viscous fluid containing most of the intracellular proteins is obtained. From this the actomyosin can be precipitated by dilution with water or by the addition of certain salts. This protein is present in larger amounts than any of the others. The other intracellular proteins, that is, the proteins of the sarcoplasm, include another globulin, *globulin X*, which is second largest in amount, *myogen*, an albumin, and small amounts of a second albumin, *myoalbumin*. In

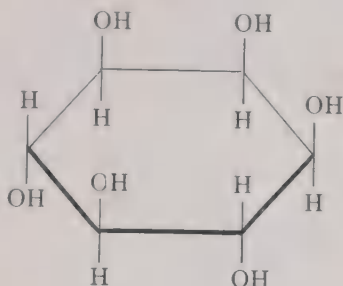
addition, *muscle hemoglobin* is present, and this gives a pink hue to some of the extracts. Muscle hemoglobin has been isolated in crystalline form. Its molecular weight, isoelectric point, and absorption bands all differ from those of blood hemoglobin, although the iron content of both is the same. There may be still other proteins present.

Actomyosin is the only protein of muscle with contractile power. Szent-Györgyi has presented evidence which indicates that this is a complex of two proteins, *myosin* and *actin*. When these are mixed they combine, and an increase in viscosity is seen. Actin has two forms, a globular and a fibrous form, while myosin occurs only in the fibrous condition. Together they form threads of actomyosin. Under the influence of  $K^+$  and  $Mg^{++}$  ions, adenosine triphosphate (ATP), a high energy compound (see page 422), is adsorbed upon the protein. When this occurs, the viscosity is observed to decrease. An enzyme which decomposes ATP, adenosine triphosphatase (ATPase), seems to be very closely associated with actomyosin, or it may even be a part of this unique protein. The effect of the enzyme is to cause a small amount of energy to be released upon the protein, initiating the contraction of the actin fibers, changing them to globules, and thus causing the actomyosin to curl and become considerably shorter. Subsequently, more energy is released to induce relaxation. It is thought that this energy is stored until the deposition of the first small amount of energy acts as a trigger to set off the next contraction.

**Muscle Lipids.**—Besides variable amounts of fat, muscle is found to contain small amounts of cholesterol and larger quantities of phospholipid. Here there are definite differences among the three types of muscle (Bloor). Smooth muscle has the greatest amount of cholesterol, cardiac muscle next, and striated muscle the least. The ratio of phospholipid to cholesterol is high for skeletal and cardiac muscle and low for smooth muscle. It is thought that these findings indicate that cholesterol has some relationship to the spontaneous muscular activity of cardiac and smooth muscle and that phospholipids are somehow concerned in the greater energy production of cardiac and striated muscle.

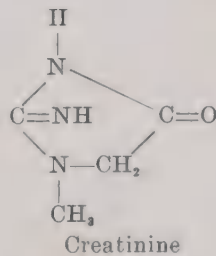
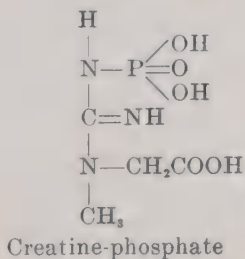
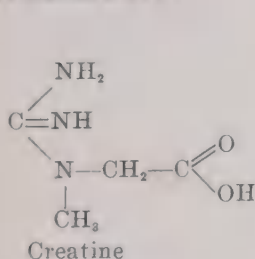
**Extractives.**—If muscle tissue is hashed and repeatedly extracted with hot water, one obtains a light tan fluid with droplets of fat floating on the surface and particles of coagulated protein suspended. When this is filtered and concentrated, a brown sticky material is left, which is commonly known as beef extract. This is composed of all the soluble inorganic salts and all of the extractives mentioned previously. The glycogen and some of the other compounds, of course, will have been hydrolyzed in the process. Aside from the small amounts of carbohydrate, amino acids, and peptides present, there is little food value in extract of beef. The idea that this has all the "strength of the beef" is quite incorrect. As a basis for soups and flavoring for other foods it adds savor and may reflexly stimulate the flow of digestive juices. Some of the constituents, such as inositol, may have specific virtues, but it must be emphasized that clear bouillon has very little nutritive value.

Some of the individual extractives deserve mention at this point. One of them is inositol,  $C_6H_{12}O_6$ , or better,  $C_6H_6(OH)_6$ . There are a number of isomers of inositol in nature. The most important one is *mesoinositol*:



The other isomers differ from this in the arrangement of the OH's and H's in space. Although not a sugar, it has a sweet taste. This is a property common to many polyatomic alcohols, including glycerol. It is widely distributed in the plant and animal kingdoms and is considered by some as part of the vitamin B complex. It is also possible that inositol is an intermediate between carbohydrates and aromatic substances. This transformation has been accomplished biologically as well as chemically. Isotopic mesoinositol also has been converted into glucose by phlorizinized (that is, diabetic) rats, although by no means efficiently. (Stetten.) In sharks and certain other fish, inositol is stored up instead of glycogen. It is thus possible that this substance plays some role in carbohydrate metabolism, either as a substitute for glycogen or as an intermediate in the transformation of one monosaccharide into another. (Fischer.)

Salts of lactic acid are present because they result from carbohydrate breakdown in muscle metabolism. Creatine and creatine-phosphate are also involved in muscle metabolism. Their formulas as well as the structurally related creatinine are:



Creatine is methylguanidine acetic acid and creatinine is its anhydride. Creatine-phosphate is a very unstable compound. In muscle contraction, creatine-phosphate plays a very important role, its great instability being partly responsible for the release of energy in a complicated series of reactions (Chapter 16). In human striated muscle there is about 350 to 400 mg. of creatine per 100 Gm., but only about one-fifth as much in nonstriated muscle. Extremely little creatinine is present—only about 5 to 10 mg. in striated and even less in nonstriated.

Another constituent which, as we shall see, is an important cog in the machinery of muscle contraction is adenosine triphosphate. When this com-



pound loses two of its phosphoric acid groups, with the release of energy, adenylic acid results. This is a nucleotide, a substance composed of a purine (or pyrimidine or other nitrogenous base), a sugar, and one molecule of phosphoric acid. Inosinic acid is another nucleotide present. The purines and pyrimidines found in extractives are probably decomposition products of nucleotides. The chemistry and significance of these products will be discussed in subsequent chapters.

At least three peptides have been isolated from muscle extracts. *Carnosine* is  $\beta$ -alanyl-histidine, and *anserine* is  $\beta$ -alanyl-methyl-histidine. The presence of a  $\beta$ -amino acid is rather extraordinary since the usual amino acids resulting from protein breakdown have the amino group in the alpha position. Carnosine has a stimulating action upon both the motor and secretory activities of the intestines and thus its presence in beef extract may be of some value. The third peptide present in muscle extractives is *glutathione*, a tripeptide, with the composition glutamyl-cysteinyl-glycine. This compound is a hydrogen acceptor and as such must have some part in tissue reactions. Just what that role is cannot be said with certainty as yet. It is found in many tissues other than muscle, especially liver, red blood cells, brain, and kidney. It is also present in the lens of the eye, and is reduced in amount when cataract occurs. The other extractives have been considered in other chapters.

**Enzymes.**—The life processes of the cell are dependent upon the functioning of many enzymes. These may be extracted, purified, and studied by the usual biochemical methods. However, some of them may also be demonstrated *within the cell* (see Fig. 83). Those enzymes demonstrable by histochemical methods include alkaline and acid phosphatase, peroxidase, tyrosinase, and dopaoxidase. Classification, action, and importance of enzymes are treated in later chapters, particularly Chapter 9.

**Vitamins.**—Vitamins also have been detected in tissue cells by histochemical procedures, and they too are considered more fully in later chapters. Vitamin A is characterized by a fading green fluorescence in ultraviolet light. It can be demonstrated in the retina, in certain human tumors, and in human skin and is present in the ovaries in variable amounts, the variations being related to cyclic changes. Niacin and riboflavin also fluoresce and thus lend themselves to similar study. Ascorbic acid is detected by observing its reducing action upon silver nitrate. It has been shown that ascorbic acid is localized in the Golgi material of cells. (Barnett and Bourne.)

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## Chapter 7

### MILK

Milk is a fluid secreted by the mammary gland for use as food for the young mammal. Consequently we must consider milk from two standpoints: first, as a secretion, and second, as a food.

**Secretion of Milk.**—Milk is secreted by the alveoli of the mammary gland. These are not present in either sex in early childhood. In the female, at puberty, proliferation of the tubules and development of the alveoli occur, and the gland, of course, increases in size. These changes occur as the result of the liberation of ovarian and other hormones. The actual secretion of milk does not occur until the end of pregnancy. The initiation of lactation may be a result of a sudden removal of the placenta and of other factors. The chief hormone involved is prolactin, a pituitary factor. There is probably also an adrenal hormone involved. Nervous stimulation induced by suckling is believed to cause the secretion of the hormones which have their continuing effect upon the mammary gland. For further discussion see Chapter 23.

#### GENERAL COMPOSITION OF MILK AND FACTORS MODIFYING IT

In addition to being used as the food of the very young, man has adopted milk, cow's milk particularly, as a nutrient for all ages. It is the most complete food found in nature and for a long period it is the only food of the young mammal. The first secretion of the mammary gland post partum differs a great deal from true milk. It is called *colostrum*. It is a yellowish, alkaline, and slightly viscid fluid. It has a higher content of total solids, the components of which are not the same as those of milk. Colostrum will coagulate on heating whereas milk will not. The lipids present in colostrum have a higher content of cholesterol and lecithins, and the fat has a higher iodine number. It seems to have a laxative action and thus may aid in bringing about evacuation of the meconium. The amount of colostrum secreted by the human being is rather small, about 150 to 300 ml. in twenty-four hours. About the third or fourth day true milk begins to be secreted and the colostrum qualities diminish steadily. For one or two weeks, however, human milk continues to retain some of the characteristics of colostrum. This is reflected in the changing composition of milk which is discussed under Period of Lactation.

Milk is an emulsion of lipids in a solution of proteins, lactose, and inorganic salts. There are also present some organic acids or their salts, vitamins, enzymes, and some undetermined constituents, including antibodies and a substance, called "lactenin," which has antibacterial properties against certain streptococci. It is not at all a fluid of constant composition, a number of factors tending to vary it. Some of these factors are: species of the secreting mammal, individual variations within the species, age, period of lactation, diet, physical and mental condition, time of day, and fraction of single nursing.

## Species

It is well known that different animals have milk of different composition. In Table XVI is given a comparison of the milk of a variety of mammals, and in Table XV it is shown how, in a general way, the proportion of protein and salts parallels the rate of growth. Animals which grow faster have food containing more protein, for soft tissue building, and more inorganic salts, for bone building. Moreover, within a given species there are differences; for example, in the composition of milk of various breeds of cattle. Holsteins produce a milk with a lower fat content than that of Jerseys and Guernseys.

TABLE XV

PROTEIN AND ASH CONTENT OF MILK OF DIFFERENT SPECIES AS RELATED TO RATE OF GROWTH\*

SPECIES	TIME IN WHICH THE BODY WEIGHT OF THE NEWBORN ANIMAL IS DOUBLED (DAYS)	PROTEIN (PER CENT)	ASH (PER CENT)	CALCIUM (PER CENT)	PHOSPHORIC ACID (PER CENT)
Man	180	1.4	0.2	0.0328	0.0473
Horse	60	2.0	0.4	0.124	0.131
Cow	47	3.4	0.7	0.160	0.197
Goat	19	3.3	0.8	0.210	0.322
Pig	18	5.2†	---	0.250§	0.537§
Sheep	10	5.5	0.9	0.272	0.412
Dog	8	7.1	1.3	0.453	0.493
Cat	7	9.5	---	---	---

\*Data chiefly from Bunge, G.: Text-Book of Physiological and Pathological Chemistry, ed. 2. (English), Philadelphia, Pa., 1902, P. Blakiston's Son & Co.

†Data from Sheffy, B. E., et al.: J. Nutrition 48: 103, 1952.

§Data from Braude, R., et al.: Brit. J. Nutrition 1: 64, 1947.

TABLE XVI

AVERAGE COMPOSITION OF MILK OF DIFFERENT SPECIES\*

SPECIES	WATER (PER CENT)	PROTEIN (N × 6.37) (PER CENT)	FAT (PER CENT)	LACTOSE (PER CENT)	ASH (PER CENT)	FUEL VALUE PER POUND (CALORIES)
Human	87.4	1.4	4.0	7.0	0.2	316
Cow	87.1	3.4	3.9	4.9	0.7	310
Goat	87.0	3.3	4.2	4.8	0.7	318
Sheep	82.6	5.5	6.5	4.5	0.9	447
Reindeer	63.7	10.3	19.7	4.8	1.5	1,078
Buffalo	82.2		7.5	4.8	0.8	
(Indian)						
Zebra	86.1	3.0	4.8	5.3	0.7	
Camel	87.1		2.9	5.4	0.7	
Mare	90.6		1.1	5.9	0.4	
Ass	90.1		1.4	6.2	0.5	
Pig†	80.1	5.8	8.2	4.8	0.9	---

\*Data, in part, from Farmer's Bulletins Nos. 363, 1909, and 1705, 1933.

†Data from Braude, R., et al.: Brit. J. Nutrition 1: 64, 1947.

It will be noted that goat's milk has about the same composition as cow's milk. The proportion of lactalbumin is slightly higher and the amounts of thiamine and riboflavin are stated to be higher also than the corresponding values for cow's milk. The curd "tension," i.e., toughness, is lower; this fact and also the somewhat greater resemblance to the distribution of proteins in human



milk has led to many claims of superiority of goat's milk over cow's milk for infant feeding. However, almost the only indication for its use is for babies who are allergic to the proteins of cow's milk.

### Individual Variations, Age

There is some individual variation in the milk of cows of the same breed and of the same cow from day to day. Consequently, the mixed milk from a herd is bound to be more uniform in composition than the milk of a single cow. Furthermore, the effect of any deleterious change in composition of the milk of a single cow, as a result of any factor whatever, is minimized by dilution with the milk of many others. This is one reason why the milk distributed by large dairies is so uniform in composition.

The same individual variations occur among human beings. Some mothers produce milk of poor quality or small volume, although most women are quite capable of nursing their babies adequately. The total volume secreted depends upon the demands of the infant and the secretory capacity of the mammary glands. In some instances, wet nurses have been found to secrete more than a gallon of milk a day. Young mothers, as a rule, secrete more milk than older ones; this does not appear to be related to the fact that there are more primiparas (first births) among the young mothers, but is probably because of youthful health and vigor. Milk secretion is under hormonal control. Undoubtedly this accounts for some of the normal variations, particularly that variation due to age. Pronounced effects upon lactation are observed in certain types of endocrine dysfunction. For example, milk secretion may persist for an exceptionally long period after childbirth in cases of acromegaly.

### Period of Lactation

Since the early secretion, colostrum, has quite a different composition from true milk, and since it is claimed that milk retains some of the characteristics of colostrum for one or two weeks, it would be expected that analyses of milk during the first two weeks would be variable. In fact, human milk continues to change for at least eight weeks. In Table XVII are given the results of an investigation of this subject; the same results compared with the average change in the weights of the infants are shown in Fig. 17. After the third

TABLE XVII  
COMPOSITION OF HUMAN MILK AT DIFFERENT PERIODS\*

TIME	NO. OF CASES	PROTEIN			SUGAR			FAT		
		MINI- MUM (PER CENT)	MAXI- MUM (PER CENT)	AVER- AGE (PER CENT)	MINI- MUM (PER CENT)	MAXI- MUM (PER CENT)	AVER- AGE (PER CENT)	MINI- MUM (PER CENT)	MAXI- MUM (PER CENT)	AVER- AGE (PER CENT)
5 days	88	1.45	2.83	2.00	4.62	7.37	6.42	0.9	8.2	3.2
9 days	88	1.12	2.65	1.73	4.76	7.65	6.73	1.6	7.1	3.7
3-4 wk.	35	1.03	1.79	1.37	6.17	7.89	7.11	1.4	6.1	3.6
5-6 wk.	32	0.98	1.57	1.30	5.97	8.33	7.11	1.3	7.6	4.0
7-8 wk.	14	1.04	1.40	1.21	6.25	7.83	7.11	1.1	7.0	4.0

\*From Bell, M.: J. Biol. Chem. 80: 239, 1928.

week, the lactose content remains constant and a little later the fat also levels off. The very first colostrum secreted is extremely high in protein, diminishing considerably during the next few days, and the amount of essential amino acids available is correspondingly high. (Miller and others.) Apparently the infant's need for high protein is most acute during the first two weeks of growth. Later it requires a higher percentage of the energy-forming foods—fats and carbohydrates.

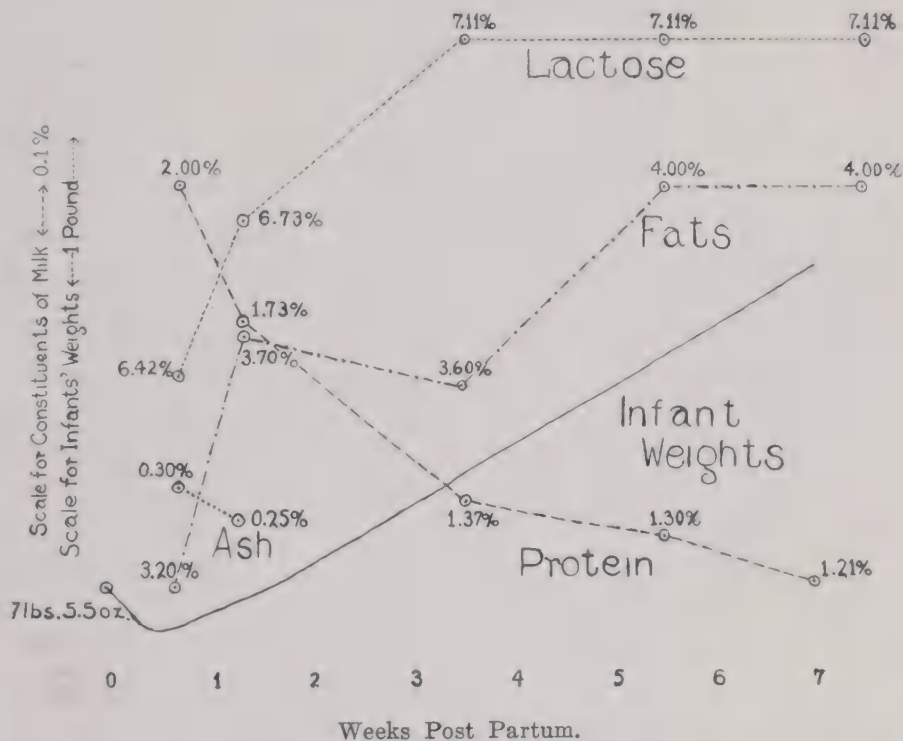


Fig. 17.—Average composition of human milk at different periods compared with the average weight curve of the infants. The different constituents are plotted on different base lines but the scale is the same in all cases. (From Bell, M.: *J. Biol. Chem.* 80: 239, 1928.)

### Diet

The diet of the mother, or other lactating animal, has some influence upon the amount and quality of the milk but not as much as one would expect. Feeding more liberally than the accepted standards results in slight changes in volume and composition. During the early days of lactation, a higher carbohydrate diet tends to increase the volume somewhat and also the lactose. High protein also increases the volume secreted, while a high fat diet forces the fat up and the volume down. However, these changes are not of great significance. The vitamin content may be improved by feeding larger amounts; this is particularly true in the case of vitamin D. A poor nutritive condition of the mother influences the quantity of the milk secreted. Courtney has shown that inadequate diets result in fluctuations of the inorganic constituents, calcium and magnesium being decreased usually, while potassium, sodium, chlorine, and phosphorus are irregularly influenced. In other respects, however, no marked changes have been noted.

### Physical and Mental Conditions, etc.

It is self-evident that a poor state of health will be reflected in an inadequate milk secretion. Either the quality or the quantity may suffer. If the mother is in good health, her milk will usually "agree with" the baby. This does not mean that the milk is necessarily adequate nutritionally. The most usual difficulties are too low or too high fat content, insufficient vitamins in the mother's diet and, consequently, in her milk, and a sensitivity to certain proteins in the milk which are traceable to the mother's diet.

The amount of milk secreted is diminished by excessive physical work, and emotional disturbances have a similar effect; e.g., excitement, worry, and fear. There is a greater volume secreted at night than during the day. The most characteristic analytical figures are said to be obtained at 9 or 10 A.M.

### Fractions of a Single Nursing

During a single nursing the mammary gland begins by secreting milk richer in proteins and poorer in fats. As secretion continues, the protein content diminishes and fat increases so that the last fraction has a composition just the opposite to the first. Consequently, if the physician wishes to get an accurate quantitative idea of the composition of a mother's milk, the entire contents of one breast should be obtained. If this is not practicable 1 ounce should be obtained before and 1 ounce after nursing and the two should be mixed.

Human milk may be obtained by simple manual expression, which is said to be more efficient than the use of breast pumps. (Davies.) Breast pumps vary from the simple funnel-shaped glass tube with an enlargement to hold the milk, and a rubber bulb at the smaller end to produce suction, to quite complicated machines. A water-power breast pump is also available. The electric pump is frequently used in hospitals. It gives rhythmic suction and, since compression of the breast occurs at the same time as suction, is very effective.

## COMPOSITION OF HUMAN AND COW'S MILK

In Table XVIII is shown a comparison of the composition of human and cow's milk given in two ways: first, the range of values ordinarily found, and second, in "round numbers," which represent the usual approximate values. The outstanding differences are a greater concentration of lactose in human

TABLE XVIII  
COMPARISON OF HUMAN AND COW'S MILK

	TOTAL PROTEIN (PER CENT)	LACTOSE (PER CENT)	FAT (PER CENT)	ASH (PER CENT)
Human milk				
Range	1.0-2.8	4.6-8.3	0.9-8.2	0.25-0.30
Round numbers	1.4	7.0	4.0	0.25
Cow's milk				
Range	2.1-6.4	2.1-6.1	1.7-6.5	0.35-1.21
Round numbers	4.0	5.0	4.0	0.75

than in cow's milk, with lower concentrations of total protein and ash. The percentage of fat is about the same in cow's and human milk, but cow's milk



is generally brought to a constant composition of about 3.9 per cent at the dairy where the milk is pooled and analyzed. The legal requirement is usually 3.25 per cent.

Besides these variations in general composition several special points should be noted. The distribution of the three milk proteins is quite different. In cow's milk the casein is greater in amount than the albumin-globulin fraction, while in human milk the albumin-globulin fraction is slightly greater. The inorganic constituents are about the same qualitatively but not quantitatively. Although human milk contains slightly more iron than cow's milk, this is not sufficient for the baby's day-to-day needs. Nature has provided for this by having the infant come into the world with a store of iron—enough to last until the infant can obtain iron in foods other than milk. The Ca and P are sufficient to provide for bone growth and tissue requirements, but they are not present in excessive amounts.

Breast feeding may be of advantage from another standpoint; i.e., because of the passive transfer of immune bodies from the mother into the milk. It has also been claimed that human milk contains an antipoliomyelitis substance.

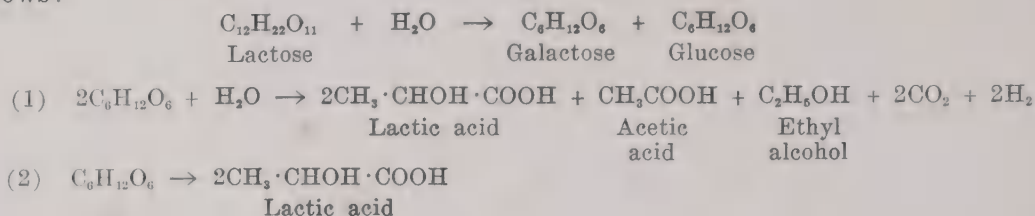
### Physical Characteristics and Reaction

Milk is a white to yellowish white fluid having a specific gravity varying from 1.026 to 1.036 (cow's and human). Since the specific gravity of fat is less than 1.000, a high fat (cream) content will tend to lower the specific gravity. "Watering," or diluting milk with water, will, of course, have the same effect. Consequently, the determination of the specific gravity will aid very little in estimating the composition of milk. The reaction is usually faintly acid, with a pH of 6.6 to 6.9. The color is partly due to the calcium salt of casein, which is bluish-white in solution, and partly to the emulsified fat. The yellowish color often observed is derived from pigments in the food which dissolve in the fat. According to Palmer and Eckles, these are carotene and xanthophyll, chiefly the former. They cannot be synthesized and if absent from the diet, similar pigments present in the body fat may be drawn upon. By changing feed, all degrees of color may be observed in butterfat, from almost colorless to a very deep yellow. The color of whey is probably due to the presence of lactoflavin, identical with riboflavin.

### Lactose

Lactose apparently occurs only in milk. Moreover, galactose is not found free in nature but is found in combination. However, not enough galactosans or other galactose-containing substances are ingested by the lactating mother to furnish the requisite amount of galactose to form lactose. Therefore, it seems certain that lactose is formed from glucose by the mammary gland. This means that one molecule of glucose must be converted to one of galactose and joined to another of glucose. Just how this is accomplished is not known. Furthermore, since the amount of glucose in the blood is insufficient to account for all the lactose, the suggestion has been made that it arises, in part, by gluconeogenesis from amino acids in the mammary gland. (See page 375.)

If milk is permitted to stand long enough at ordinary temperatures it sours. This is called lactic "fermentation," but since it is not always accompanied by evolution of a gas, the more accurate term would be "lactic acid production." It is brought about by certain bacteria and the reaction may result in (1) about 50 per cent production of lactic acid or (2) about 100 per cent, depending upon the type of organism present. The reactions would be, perhaps, as follows:

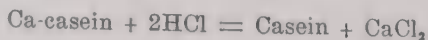


Although lactose is the natural milk sugar, and peculiar to milk, the question arises, does it have special advantages in infant nutrition? Brody and Sadhu feel that the spatial configuration of lactose, and especially of its constituent galactose, gives it "unique nutritional properties." The sugar level in milk tends to vary directly with the weight of the adult brain. Man has the largest brain, in proportion to the body weight, of all animals, and human milk has the highest percentage of milk sugar. This may be related to the glycolipids of brain, which, it will be remembered, usually contain galactose. Moreover, glucose is the only fuel normally used by the brain in its activities, and it is conceivable that during brain formation the more stable galactose being less likely to be oxidized is more suitable as a building material. Infants may be less able to synthesize galactose than adults, hence its presence in their food may be highly desirable from this standpoint. However, physicians seldom write formulas containing lactose. If there is a peculiar need for lactose, there seems to be enough present in the milk which is invariably a large part of the formula.

### Proteins

The proteins of milk are casein, lactalbumin, and lactoglobulin. These proteins, particularly casein, are peculiar to milk. They are manufactured by the mammary glands from the amino acids present in the blood. This has been proved by the intravenous injection of valine, lysine, and glycine, labeled with  $\text{C}^{14}$ , into lactating rabbits, and finding the labeled carbon in the milk proteins. (Campbell and Work.) The loss of protein during lactation has no deleterious effect upon the mother's blood proteins which are maintained at normal levels. Of course, the lactating woman must have a plentiful supply of proteins of good quality in her diet in order to provide the amino acids necessary to build these proteins.

Casein is a phosphoprotein. It is insoluble at its isoelectric point, pH 4.6, but since the pH of milk is nearly 7.0, it is undoubtedly present as a salt, calcium caseinate. On acidification casein precipitates.



In the souring of milk the same reaction occurs. Rennin precipitates casein also, and, as stated previously, pepsin and other proteolytic enzymes will do the same. In these cases, it must be remembered, the process is a digestion and may occur at a pH other than 4.6. An early digestion product of casein, paracasein, combines with the calcium present to form an insoluble salt, calcium p-caseinate. This can be demonstrated by adding some oxalate to milk and obtaining by filtration the decalcified milk. Rennin, if added to this decalcified milk, will not clot it, whereas it will clot the untreated milk in a few minutes, but subsequent addition of a soluble calcium salt, in excess, will bring down a clot. Clotted milk, or junket, is frequently used in the American dietary.

According to Linderström-Lang, casein is not a single protein but is a group of three proteins. One of these acts as a protective colloid for the other two which would otherwise precipitate out. An attack by a proteolytic enzyme on this protective colloidal protein renders it incapable of protecting the other two, and if calcium ions are present, and pH and temperature favorable, they precipitate then as calcium-p-caseinate.

Lactalbumin and lactoglobulin are typical albumin and globulin, respectively. The relative proportions of the three proteins vary among different species. As mentioned, this variation is quite marked in the case of cow's and human milk. Lactalbumin and lactoglobulin are both coagulable proteins and will coagulate when isolated from milk by suitable separation methods. As present in milk, they do not coagulate on heating because the pH is not favorable, but they are undoubtedly denatured by heat.

All three proteins are excellent biologically, containing a wide assortment of amino acids. The combined milk proteins yield all the known amino acids, essential and unessential. Table XIX indicates the content of the essential amino acids in the proteins of cow's milk, as well as the amount present in one quart. When compared with the recommended daily intake of these amino acids, it is seen that a quart of milk will provide a large proportion of the adult's daily requirement of essential amino acids. From a consideration of the amino acid content alone, human milk proteins are not nutritionally superior to the proteins of cow's milk. (Block and Bolling.) The difference

TABLE XIX  
ESSENTIAL AMINO ACIDS IN COW'S MILK

AMINO ACID	PER CENT IN MILK PROTEINS*	RECOMMENDED DAILY INTAKE	<i>One Quart of Milk Contains</i>	
		FOR MAN† GM.	GM.	PER CENT OF RECOMMENDED DAILY INTAKE
L-Tryptophan	1.6	0.5	0.5	100
L-Phenylalanine	5.7	2.2	1.9	86
L-Lysine	7.5	1.6	2.6	163
L-Threonine	4.6	1.0	1.6	160
L-Valine	5.0	1.6	1.7	106
L-Methionine	4.0‡	2.2	1.4‡	64
L-Leucine	15.0	2.2	5.2	236
L-Isoleucine	4.4	1.4	1.5	107

\*Based on Block, R. J., and Bolling, D.: Nutritional Opportunities With Amino Acids, *J. Am. Dietet. A.* 20: 69, 1944.

†Rose, W. C.: Amino Acid Requirements of Man, *Fed. Proc.* 8: 546, 1949.

‡Methionine and cystine.



in the proportion of albumin to casein explains why cow's milk forms heavy tough curds and human milk soft fine curds. Such soft fine curds are much more easily and rapidly digested.

Very small amounts of free amino acids are also present in milk. These represent about 4 and 6 mg. of nonprotein nitrogen per 100 ml. of cow's and human milk, respectively. In this connection, it is interesting to note that the objectionable flavor which milk acquires on exposure to sunlight for periods of a half hour or more has been found to be due to photolysis of methionine, aided by the vitamin riboflavin. (Patton and Josephson.)

## Lipids

The fat of milk is in the form of very small globules. There is no appreciable difference in the size of the fat particles in human as compared with cow's milk, although there may be slight variation in the fat globules obtained from different individuals. Most of milk fat of cow's milk consists of the triglycerides of palmitic, oleic, stearic, myristic, and other higher fatty acids, but a small amount, about 10 per cent, is composed of the triglycerides of butyric, caproic, caprylic, and other fatty acids with short carbon chains. The latter include several volatile fatty acids; in this respect, milk fat differs from other fats formed in the body. Small amounts of cholesterol, phospholipids, and free fatty acids are present. Human milk fat differs from bovine in that few or none of the fatty acids present have chains shorter than ten carbons. Oleic acid occurs in largest amount, palmitic next, lauric, myristic, stearic, octadecadienoic in smaller amounts, and still smaller quantities of a considerable number of long-chain acids. The fat of human milk resembles human body fat much more than it does typical milk fat of other species. (Brown and Orians.) Milk fat probably is derived from glycerol and the fatty acids of the blood, although the phospholipids and the cholesterol esters have been considered their precursors by various authorities until recently.

Since the fat globules are lighter than water, they rise to the top to form cream. Commercially, the fat of milk is its most valuable constituent, marketed as cream and butter and entering into the composition of cheese. The percentage of fat in milk often determines the price of milk to the farmer. It is also an index of the nutritional value of mother's milk. Consequently the analysis of milk for fat is of considerable importance to farmers and dairymen, as well as to food and health authorities, and, of course, to the physician. There are many methods of accomplishing this, but the quickest, easiest, and almost universally adopted method is the Babcock procedure. This can be used not only for cow's milk, but, with modifications, also for human milk, cream, skim milk, ice cream, etc.

**Babcock Method.**—In the Babcock method, measured amounts of milk and concentrated sulfuric acid are mixed in a special flask which has a narrow graduated neck. The proportion of concentrated sulfuric acid used is sufficient to cause solution of the proteins, which at first precipitate, and to remove the protective layer around the droplets of lipid material. The fats and other lipids are not destroyed or dissolved in the acid but are kept liquid by the heat generated and maintained by adding hot water. The flasks are whirled in a centrifuge and the liquid layer of fat rises in the narrow neck. Since the graduations are in per cent of fat, the result is found by reading the lowest point of the fat column and subtracting that

figure from the highest point at the top of the fat column. The centrifuge flasks vary with the type of dairy material to be analyzed; viz., cream, skim milk, etc. For human milk a centrifuge tube with a narrow graduated neck is employed because of the limited volume usually available, and slight modifications in technique are used.

The amount of cholesterol in cow's milk is approximately 11 mg. per 100 ml., all of which is free cholesterol. (Nataf and others.) The cholesterol content of the milk does not seem to bear any relationship to the level of this lipid in the cow's blood serum.

### Ash

The inorganic salts of milk include chiefly calcium, potassium, and sodium salts of hydrochloric and phosphoric acids. Potassium is present in larger amounts than sodium. Other elements present in milk include magnesium, sulfur, iron, copper, iodine, and zinc (see Table XX). Although some elements are present only in traces, it must not be assumed that they are of no value. Only minute amounts of copper, for instance, are needed in hemoglobin formation. Iron, it is true, is present in too small an amount in milk to warrant the exclusive use of this food in later childhood. Iron-containing foods should supplement the diet. Milk is, however, the most practical source of calcium and a very good source of phosphorus. Both of these are needed by all cells, but particularly for bone and tooth structure.

TABLE XX  
COMPOSITION OF ASH OF MATURE HUMAN AND COW'S MILK\*

	HUMAN MILK (MG. PER 100 ML.)	COW'S MILK (MG. PER 100 ML.)
Total ash	210	710
Calcium	34	126
Chlorine	43	100
Magnesium	4	13
Phosphorus	16	99
Potassium	55	138
Sodium	15	58
Sulfur	14	30
Bromine	0.91	0.02
Copper	0.04	0.03
Iodine	0.01†	0.02
Iron	0.21	0.13
Rubidium	1	Trace
Zinc	0.66	0.35
Aluminum, Barium, Boron, Chromium, Lead, Lithium, Manganese, Molybdenum, Silver, Strontium, Titanium, Vanadium	Traces	Traces

\*Data from The Composition of Milks, Bull. Nat. Research Council, No. 119, 1950; compiled by Macie, I. G., Kelley, H., and Sloan, R.

†Skimmed milk.

### Vitamins

Milk contains most of the vitamins in greater or smaller amounts. It is quite deficient in vitamins C and D and rather low in the B vitamins, except riboflavin. Apparently mother's milk cannot be enhanced with vitamin D to any significant degree by feeding fish liver oils containing large amounts of the

TABLE XXI

## VITAMIN CONTENTS OF FRESH MATURE HUMAN AND COW'S MILK\*

VITAMIN (CONCENTRATION PER 100 ML.)	HUMAN MILK	COW'S MILK
Vitamin A, $\mu\text{g}$	54	37
Carotenoids, $\mu\text{g}$	32	39
Vitamin D, U. S. P. units	0.4 to 10†	0.5 to 4†
Vitamin E, mg.	0.66	0.06
	0.10-0.48	
Vitamin K, Dam-Glavind units	26	100
Ascorbic acid, mg.	4.4	1.8‡
Biotin, $\mu\text{g}$	0.4	3.5
Choline, mg.	9	13
Folic acid, $\mu\text{g}$	0.01-0.22§	0.02-0.4§
Inositol, total, mg.	39	13
Nicotinic acid, $\mu\text{g}$	172	85
Pantothenic acid, $\mu\text{g}$	203	350
Pyridoxine, $\mu\text{g}$	11	48
Riboflavin, total, $\mu\text{g}$	46.9	158‡
Thiamine, total, $\mu\text{g}$	15	42
Vitamin B <sub>12</sub> , $\mu\text{g}$	0.01-0.16§	0.32-1.24§

\*Data, except where otherwise noted, from The Composition of Milks, Bull. Nat. Research Council, No. 119, 1950; compiled by Macy, I. G., Kelley, H., and Sloan, R.

†Data from Lawrence, J. M., Herrington, B. L., and Maynard, L. A.: Am. J. Dis. Child. 70: 194, 1945.

‡Very large losses of ascorbic acid and riboflavin may occur during the processing and delivery of cow's milk. Small losses of thiamine may also occur. The other vitamins, in most cases, are known to be stable.

§Data from Collins, R. A., et al.: J. Nutrition 43: 313, 1951.

||Harris P. L., Quaife, M. L., and O'Grady, P.: J. Nutrition 46: 459, 1952.

vitamin during pregnancy. In order to enrich the milk with vitamin D, this factor must be taken just prior to or during lactation. (Polskin.) Macy and co-workers have shown that the content of vitamins A and C also can be increased in human milk if the lactating woman is fed large amounts of these vitamins in a multiple vitamin supplement. Milk is an excellent source of vitamin A, riboflavin, and pantothenic acid. Babies usually are given orange juice or tomato juice to furnish additional C, or ascorbic acid itself, and there are various methods of increasing their vitamin D intake. Pregnant and lactating women on high milk diets also require additional vitamins C and D as well as the B complex. Table XXI gives a comparison of the vitamin contents of human and cow's milk. Since the amounts of the different vitamins vary with diet, exposure to sunlight, and other factors, these figures are to be considered as typical rather than absolute values. It will be noted that in several instances there are marked differences, with the human milk usually, but not always, the richer of the two.

**Enzymes.**—A catalase, a peroxidase, and a phosphatase are present in milk. It is doubtful whether these have any significance, but the presence of an active enzyme is evidence that the milk has not been pasteurized or sterilized.

**Sterilization and Pasteurization.**—It is practically impossible to obtain sterile milk from the mammary gland of a cow or of a woman unless the first portion obtained, which washes out the organisms present in the ducts of the nipple, is discarded and bacteriological technique is employed. Modern dairy practice, with extreme emphasis on cleanliness and the use of sterile containers,



has reduced the bacterial count greatly. Nevertheless, public health authorities do not recommend raw milk for human consumption. Milk is seldom sterilized for ordinary sale and use but is commonly rendered safe by pasteurization. In pasteurization the milk is heated to 60 to 63° C. and is held at that temperature for thirty minutes or is kept at 72° C. for fifteen seconds. It is then cooled rapidly and kept cold until delivered. Pasteurized milk is free from pathogenic bacteria, although it usually contains other germs. The pathogenic organisms which cause tuberculosis, undulant fever, diphtheria, the streptococcal diseases, septic sore throat, and scarlet fever, typhoid fever, dysentery, and others are destroyed by this treatment. Pasteurization, of course, is not an insurance against subsequent contamination. In order to prevent bacteria from multiplying, all milk should be kept at 10° C. or below. In pasteurization some precipitation of calcium phosphate occurs and about 10 per cent of vitamin B<sub>1</sub> and 30 per cent of vitamin C are destroyed. Boiling raw milk from one to three minutes is somewhat more effective than pasteurization in so far as the bacteria are concerned. It has the same effect upon the vitamins and produces a film which contains a small amount of the nutritive elements (see next paragraph). The flavor of boiled milk is different from pasteurized and is not relished by some people. For infant feeding, boiled milk, even if previously pasteurized, or milk heated for twenty minutes in a double boiler is recommended by most pediatricians. True sterilization of milk usually requires heating to 100° C. on several successive days or a single application of heat at 120° C. for thirty minutes under fifteen pounds pressure.

**Film Formation.**—When milk is heated to about 60° C. a film, or “skin,” forms on the surface. Upon removing the pellicle, cooling, and reheating, a second film forms. If the heating is done in a closed vessel, no such skin forms even at 100° C. The skin consists of protein, calcium phosphate, and some enmeshed fat globules. The mechanism of this phenomenon is rather complicated. At the air-liquid interface there occurs a concentration of the proteins, because the water evaporates faster than it can be replaced by diffusion. The proteins thus form a semisolid layer in which fat globules are enmeshed. This retards the diffusion of water still more. There then occurs an irreversible precipitation of proteins, the casein micelles being destabilized, and the albumin and globulin being denatured or coagulated to some degree. If the boiled milk is to be used for infant feeding, the film may be removed and discarded. The loss in nutritive value is slight.

**Passage of Foreign Substances Into Milk.**—It is well known that cow's milk acquires distinctive and often unpleasant flavors if the cow feeds on some strong-tasting food, such as turnips, onions, garlic, or wild carrots. The volatile oil or other flavor of the food is absorbed and finally secreted by the mammary gland. The same is true of human milk and the possibility of ingestion of toxic substances by the infant via the mother's milk should be mentioned. Among the substances known to be secreted by the mammary gland after they have been administered to the mother are opium, morphine, alcohol, barbiturates, ergot, cascara, thiouracil, salicylates, iodides, bromides, arsenic, bismuth, antimony, zinc, lead, mercury, and iron. However, very few of these

have been found in high enough concentrations to be injurious to the infant. Barbiturates, iodides, and bromides are among these. Thiouracil is the only substance known to appear in milk at a higher level than in blood or urine. (Mautner.) Nicotine is not secreted by the mammary gland under moderate smoking conditions, and the amounts of alcohol and caffeine which may be secreted are of no importance. In cases of jaundice, neither bile pigments nor bile salts are found in the milk.

**Milk Products.**—From a commercial standpoint the fat of milk is its most valuable constituent. This is concentrated in cream and butter. If milk is allowed to stand, the cream rises and may be poured or skimmed off. Centrifuging is the commercial way of doing this. The fluid remaining is *skim milk*. From Table XXII it may be seen that skim milk has almost the same composition as

TABLE XXII  
COMPOSITION OF DAIRY PRODUCTS\*

	PROTEIN (PER CENT)	FAT (PER CENT)	CARBO- HY- DRATE (PER CENT)	WATER (PER CENT)	CAL- ORIES (PER 100 GM.)	CAL- CIUM (PER CENT)	PHOS- PHORUS (PER CENT)	IRON (PER CENT)
Milk, whole fresh cow's	4	4	5	87	69	0.120	0.093	0.0002
Milk, skim fresh	4	<1	5	91	36	0.122	0.096	0.0002
Cream, 20 per cent	3	20	4	73	192	0.086	0.067	0.0002
Cream, 40 per cent	2	35	3	59	337	0.086	0.067	0.0002
Buttermilk, churned from cream	4	1	5	91	37	0.105	0.097	0.0003
Buttermilk, cultured skim	4	<1	5	91	36	0.105	0.097	0.0003
Milk, condensed, sweet- ened	8	8	55	27	327	0.300	0.235	0.0006
Milk, evaporated, un- sweetened	7	8	10	74	139	0.316	0.244	0.0007
Milk, kumiss	3	2	6	90	265			
Milk, malted, dry	15	9	71	3	418			
Milk, powdered, skim	36	1	52	4	359	1.220	0.960	0.0030
Milk, powdered, whole	26	27	38	4	496	0.900	0.696	0.0017
Milk, whey	1	<1	5	93	27	0.044	0.035	
Butter	1	81	<1	16	733	0.015	0.017	0.0002
Cheese, cottage, skim milk	19	1	4	74	101	0.124	0.177	0.0003
Cheese, Swiss	29	31	2	34	404	1.086	0.812	0.0013
Ice cream, average com- mercial	3	15	18		219	0.08	0.06	0.002

The figures in the first five columns are given to the nearest whole number.

\*Data from Hawley, E. E., and Carden, G.: The Art and Science of Nutrition, St. Louis, 1941, The C. V. Mosby Co.

whole milk except for a low fat content. In diets designed to reduce body weight, skim milk is useful in providing calcium and good proteins, while at the same time limiting the caloric intake. It must be remembered, however, that skim milk is deficient in vitamin A but contains the same amount of water-soluble vitamins as whole milk. Cream contains the same constituents as milk but in different proportions. Ordinarily, cream is sold in two grades: 20 per cent fat in thin cream and 40 per cent in thick or whipping cream. Butter is produced by churning or agitating milk or cream. Usually it is first soured by lactic acid bacteria in order to permit the fat globules to coalesce more easily.



Since the vitamins A and D of milk are dissolved in the fat, it is evident that cream and butter are much better sources of these vitamins than is milk.

The fluid left after milk is churned in butter-making is called *buttermilk*. It differs very little in food value from skim milk, but since it has been soured, the casein has been precipitated. Buttermilk is the most popular "fermented" milk used in this country, but frequently the buttermilk sold is really "cultured" buttermilk. This is made by treating raw or pasteurized milk (skim or whole milk) with lactic acid bacteria cultures and then breaking up the curd into fine particles. Such milk may have almost the same food value as whole milk. Various other types of fermented milk are used here and abroad. Kefir, kumiss, and yoghurt are widely used in central Asia and Turkey, and acidophilus milk is marketed in this country. It is believed by some that these fermented-milk products, containing as they do, tremendous numbers of lactic acid-producing bacteria, tend to replace the intestinal bacteria with these lactic acid bacteria. This, it is assumed, is favorable to health. There is no doubt that the fine soft curd of these fermented milk products is more easily digested than the tough curd which usually forms when cow's milk reaches the stomach, but whether they promote the growth of a more favorable flora in the large intestine is still an open question. In infant feeding the fermented milks are sometimes used, especially in cases of pylorospasm. It is said that more rapid opening of the pylorus is promoted.

When milk is clotted by rennin or acid, it becomes *curds* and *whey*. The whey, which is the fluid which may be pressed or squeezed out, carries with it a large proportion of the lactose, some of the albumin and globulin, and a part of the water-soluble vitamins and minerals. The solids, or curds, become *cottage cheese*. Cheese may be made from cream, whole milk, or skim milk. The various types and flavors depend upon the type of milk, the method and degree of curing or "aging," and the various organisms which bring about curing. Some particular varieties are prepared only in certain localities where, it is asserted, the required organisms thrive. Cheese is an excellent source of protein and usually a good source of fat, calcium, and other minerals.

Homogenization is the process of reducing the size of the fat globules of milk by physical means. This is accomplished by forcing the milk through a very small aperture under high pressure. The fat globules of *homogenized milk* are approximately one-sixth of their original diameter. Such fat does not rise as cream when the milk stands. Having a greater surface area the fat of homogenized milk should be more rapidly digested by lipases. Furthermore, with such an increased area of fat the amount of protein encasing the globules is increased. This tends to make less protein available in solution when clotting occurs and a softer curd is formed. Cream may also be homogenized.

The large proportion of water in milk, over 85 per cent, has led to several methods of concentration in order to have less bulk for transportation and at the same time to improve its keeping qualities. *Evaporated milk* is cow's milk, which has had about 60 per cent of the water removed, and is then homogenized and sterilized in hermetically sealed cans. It contains no added substance. *Condensed milk* is reduced to about the same concentration as evapo-



rated milk, but sugar is added which acts as a preservative. Evaporated milk is heated to a higher temperature than condensed milk to ensure preservation. *Dry milk* contains no added substance. It may be prepared from whole, half skim, or skim milk. The two principal methods of drying are the roller process and the spray process. In the former, thin layers of milk pass over heated rollers in vacuo and the dried milk is scraped off the rollers. In the spray process the partially evaporated milk is sprayed into warm drying chambers. Some dried milk products are irradiated to increase their vitamin D value. The food values of the concentrated and dried milks are comparable with the milks from which they were obtained, the only loss being in the heat-labile vitamins. The flavors of these products vary somewhat but are agreeable to most people. The high sugar content of condensed milk must be remembered in advising its use in infant feeding.

The small amount of vitamin D in milk produced by cows on ordinary rations and the fact that many physicians consider vitamin D best assimilated when consumed dispersed in milk have created a demand for *vitamin D milk*. This is pasteurized, pasteurized-homogenized, evaporated, or dried milk which has been enriched with vitamin D. To meet the requirements of acceptance by the Council on Foods and Nutrition of the American Medical Association, vitamin D milk must contain not more than 400 U.S.P. units of vitamin D per quart. Vitamin D milks may be produced by (1) mixing into the milk a purified concentrate of natural vitamin D or a pure crystalline vitamin D<sub>3</sub> or D<sub>2</sub>, (2) irradiating the milk, or (3) feeding irradiated material (usually yeast) to the cow. The latter two methods are rapidly disappearing from commercial practice. The first method makes possible a milk with a vitamin D content of 400 U.S.P. units per quart. The vitamin D content of milk may be increased by adding two activated sterols: 7-dehydrocholesterol, found in animal fats, or ergosterol found in plants. The 7-dehydrocholesterol is changed to vitamin D<sub>3</sub> when irradiated, the ergosterol is changed to vitamin D<sub>2</sub>. Many pediatricians, though not all, prefer vitamin D<sub>3</sub> to vitamin D<sub>2</sub>, perhaps because calciferol (D<sub>2</sub>), in large doses, has been found to be slightly more toxic in animal experiments.

### Nutritive Importance of Milk

Although the nutritive importance of milk has been stressed frequently in this chapter, a few additional points may be taken up. Milk is the ideal food for the infant, an excellent one for the growing child, and a very good food for the adult. Its proteins are among the best biologically, its carbohydrate and fat easily digested and assimilated. It also contains 0.1-0.2 per cent citrate, the significance of which is not known. Regarding minerals, it is a dependable source of calcium and phosphorus. Vitamins A and B<sub>2</sub> are present in large amounts and other vitamins to a lesser extent. Nutritionists generally agree that growing children and adults should drink more milk. If one quart of milk is included in the daily dietary, more than the usual calcium requirement is provided as well as two-thirds of the phosphorus, one-third of the vitamin A, and the full quota of riboflavin. A pint a day will go a long way toward meeting requirements and should be the minimum daily intake of each individual.

However, milk should not be the sole, or even the main, article of diet for adults. To meet the energy requirements of an adult a very large volume would be needed, putting an undue strain upon heart and kidney. It would also provide an unnecessarily large amount of protein. Milk is deficient in some of the vitamins, but its greatest defect is its low iron content. Animals fed on an exclusive milk diet develop a nutritional anemia. Von Haam and Beard have shown that the type of milk used makes a great difference. Goat's milk is more likely to produce this anemia in rats than is cow's milk, and human milk has no deleterious effect. Nevertheless, breast-fed babies occasionally develop this type of anemia, "the nutritional anemia of infancy," particularly those prematurely born.

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## Chapter 8

### BLOOD

The cells of the tissues of the body are in contact with body fluids which, in turn, are in equilibrium with the fluid portion of the blood. In the course of the development of the higher animals this relationship of the organism to its environment was even more direct. The unicellular organisms were surrounded by sea water, and probably their mineral content was quite similar to it, both qualitatively and quantitatively. Lakes which are of Pre-Cambrian origin have a far different distribution of ions than is found in ocean water today, and this, together with other data, indicates that sea water in early geologic ages differed from modern sea water considerably. Thus potassium was probably present in greater concentration than sodium, whereas the reverse is true in present-day sea water.

As time went on, the electrolyte content of ocean water gradually changed because of the weathering of rocks, solution of minerals, precipitation of salts, and consequent shifting of equilibria. Such changes in the environment would make stabilization of conditions within the cell difficult. Apparently the evolution of the nucleus provided a mechanism for maintaining internal equilibrium, despite a constantly changing external medium. This change was evidenced by an increase in sodium and decrease in potassium ions. Although both sodium and potassium salts were deposited on land by rainfall, wind, and inundations, the sodium salts were washed back in larger proportion than the potassium, because the latter formed insoluble salts with silicon and aluminum, and because the living organisms retained more potassium than sodium. Thus sea water gradually changed until, in early Cambrian times, when many multicellular organisms had evolved and the vascular tube had been sealed, the sea water, and hence the circulating tissue fluids, contained more sodium than potassium, less calcium, and still less magnesium. This early Cambrian sea water closely resembles modern mammalian blood serum in electrolyte content (MacCallum).

#### Functions

Blood has a multitude of functions, all of which are highly necessary to health and many to life itself. This is so apparent to the layman that he often becomes unduly alarmed at the loss of even a small quantity of it. Since blood makes up one-eleventh to one-twelfth of the body weight, it is evident that a person weighing only 120 pounds has about 10 pounds of blood and the loss of a pint, approximately a pound, would not be very serious. Indeed thousands of persons have donated considerable amounts of blood both in peacetime and in wartime for whole blood transfusions and for the production of plasma. However, although single donations do not ordinarily cause more than slight discomfort, they should not be repeated too frequently, and evidence of complete blood regeneration should be demanded.



Among the chief functions of blood are the following:

1. Transportation of foods, or the products resulting from their digestion, from the intestines and the chyle ducts to the tissues, from the liver to the tissues and back to the liver, and from one tissue to another.
2. Exchange of the respiratory gases between the lungs and the tissues.
3. Transportation of waste products arising in metabolism, such as urea and uric acid, to the kidneys, skin, intestines, or liver for excretion.
4. Distribution of hormones, enzymes, vitamins, and other substances, by which the effective agent is brought almost instantaneously to the organ or tissue which is to be stimulated or inhibited.
5. Protection against microorganisms; leucocytes, antitoxins, and other factors aid in combating these foreign invaders.
6. An aid in acid-base balance.
7. An aid in water balance.
8. Heat regulation of the body which is controlled largely by the shifting of the blood to or from the surface of the body.

Some of these functions will be discussed in other chapters and one (No. 5) belongs in the province of bacteriology and immunology.

### General Composition

Circulating blood consists of a fluid portion, the plasma, and the formed elements. The former is 55 to 60 per cent by volume of whole blood. The formed elements are the red blood cells, or erythrocytes, the white blood cells, or leucocytes, and the blood platelets. It is usually stated that the average number of red blood cells in the human being normally is 5,400,000 per cubic millimeter for men and 4,900,000 for women, but higher figures are not uncommon. There is a slight fluctuation in the red cell count during the day; it is lowest during sleep, increases on awakening, and continues to increase during the rest of the day. Persons living at high altitudes usually have a higher count than those living at sea level. High red cell counts also follow muscular exercise, emotional excitement, and increased atmospheric temperature. These are temporary changes, resulting from a flow of concentrated blood from the spleen. Any condition which tends to lower the oxygen content of the blood causes an increase in the number of erythrocytes. On the other hand, conditions which increase the oxygen of the blood cause a decrease in the red cell count. High barometric pressure is an example. Pathologically, increases and decreases in the number of red cells frequently occur. A condition in which there is an increased red cell count is called a polycythemia; an anemia is a condition in which there is either a lowered red cell count or a subnormal concentration of hemoglobin.

If blood is removed from the artery or vein, it will clot or coagulate in a few minutes. The whole mass becomes gelatinous. If undisturbed, a clear straw-colored fluid is gradually squeezed out; this is *serum*. In this case the formed elements have become enmeshed in the clot. Blood plasma may be separated from the formed elements if, immediately after the blood is obtained, it is placed in paraffin-lined vessels and centrifuged in a cold atmosphere. There

is thus obtained a fluid, which is also clear and straw colored, and which will clot in a short time, gradually squeezing out serum from the clot in the process.

$$\begin{aligned}\text{Whole blood} - \text{Formed elements} &= \text{Plasma} \\ \text{Whole blood} - (\text{Formed elements} + \text{Clotting factors}) &= \text{Serum} \\ \text{Plasma} - \text{Clotting factors} &= \text{Serum}\end{aligned}$$

Clotting, of course, is a process which protects the individual from excessive loss of blood. For purposes of chemical analysis or other studies, it is often inconvenient to have the blood clot; therefore methods of preventing clotting are used. Whipping, or defibrinating blood, will accomplish this. Blood is beaten with feathers, or twigs, or is shaken in a bottle with glass beads. The blood fibrin, which forms the clot, elings to the foreign object and the fluid blood remains. However, as can readily be seen, this is not really preventing clotting, but causing clotting and removing the fibrin conveniently. Defibrinated blood is whole blood minus the clotting factors. Addition of soluble oxalates, citrates, or fluorides to blood as it flows from a blood vessel will prevent it from clotting in vitro by precipitating calcium or changing it to an un-ionized form, since calcium ions are necessary for clotting. Hirudin, a substance derived from the salivary gland of the medicinal leech, and heparin, from liver and other tissues, will do the same thing and will also prevent blood from clotting if they are injected into the animal. The injection of toxic doses of protein digests ("proteoses and peptones") will likewise prevent the blood from clotting, but they will not act upon blood in vitro. As indicated, both cold and paraffin-lined containers inhibit, but do not prevent, clotting.

### Physical Characteristics

Arterial blood is a bright crimson in color; venous blood is a darker red but is not purple or blue. Blood is more viscid than water, the viscosity being due to the many corpuscles present and to the high protein content. The pH is approximately 7.4, with an extreme normal range of from 7.3 to 7.5. The mechanisms for maintaining this constancy of reaction are varied and will be taken up in a later chapter. The specific gravity of blood ranges from 1.035 to 1.075. This may be determined clinically by Hammerschlag's method, which simply consists of letting drops of blood fall into mixtures of chloroform and benzene of varying proportions. If the drop does not rise or sink in one mixture, it evidently has the same specific gravity, which can easily be determined by a hydrometer.

**THE COPPER SULFATE METHOD.**—Various modifications of Hammerschlag's method have been devised because of the difficulty of preparing these mixtures and because, once prepared, they are likely to change in specific gravity due to the differential evaporation of the components. A practical "copper sulfate method" for the determination of the specific gravity of whole blood or plasma has been described by Phillips, Van Slyke, and co-workers and has found wide application. A series of solutions of copper sulfate of increasing concentrations is used instead of the chloroform-benzene mixtures. Each drop of blood on entering the solution becomes encased in a covering of copper proteinate and remains as a discrete drop, without change of gravity for fifteen to twenty seconds, during which its rise or fall reveals its gravity relative to the test solution. The test solutions may be prepared



from a saturated solution of copper sulfate. The copper sulfate solution automatically cleans itself; that is, after the test is completed the drop settles to the bottom as a precipitate. Then it may be used again and again, since its specific gravity is not appreciably affected until a large number of tests have been made. For accurate work, a series of copper sulfate solutions graded at intervals of 0.001 in specific gravity is used. Thus, twenty solutions cover the plasma range from 1.015 to 1.035 and forty cover the entire blood range from 1.035 to 1.075. It has been shown that the total red cell volume and hemoglobin concentration can be ascertained if the specific gravities of whole blood and plasma are known and that the protein content can be computed from the specific gravity of plasma (Ashworth and Tivertt; Moore and Van Slyke). For these estimations simple line charts have been prepared. It should be mentioned that whole blood may be introduced directly into the copper sulfate solutions, but if an anticoagulant is required, more than the minimum amount of heparin or of oxalate should be avoided so that the specific gravity is not appreciably affected.

**THE FALLING DROP METHOD.**—The specific gravity can also be determined by the falling drop method. This is based on the fact that the rate of fall of a small sphere in a viscous fluid is a function of the size and specific gravity of the sphere, the specific gravity and viscosity of the fluid through which it falls, and the acceleration due to gravity. The method provides that the drop of serum or plasma be timed as it falls through an oil, with which it is not miscible. By using a calibrated pipette, the radius of the drops is kept constant. The specific gravity of the drop may be determined from its rate of fall in the oil, which is a mixture of xylene and bromobenzene, or a mixture of methyl salicylate and mineral oil. The rate of fall is determined by timing the duration of the fall between two etched rings on the glass cylinder containing the oil, 10 cm. apart. The specific gravity is ascertained by consulting a graph which is plotted from the falling drop time of solutions of salts whose specific gravities are known. The applications to blood plasma and serum are similar to those for the copper sulfate method.

**SEDIMENTATION RATE.**—When the blood is circulating, the movement keeps the cells quite evenly mixed. When taken from the blood vessel, the formed elements immediately begin to settle out. The blood usually clots before this can occur to an appreciable extent, and the cells are enmeshed in the fibrinous gel, but if clotting is prevented, the cells will settle out slowly, leaving a clear layer of plasma at the top. The rate of settling out, or sedimentation time, is being determined widely for clinical purposes. The blood is prevented from clotting by adding a definite amount of an anticoagulant to it. It is then drawn up into a standard tube, and the length of the column of clear plasma which is seen at the top is measured at the end of one hour. The *suspension stability* of the blood is thus determined. There are several methods in common use, and some include refinements of this general technique. Consequently the figures obtained by the different methods are not comparable. It is therefore advisable at the present time to report sedimentation rate as "normal," "fast," "very fast," or "slow." Normal men generally have a lower rate than women, and newborn infants have a very low rate. In menstruation and normal pregnancy the rate is markedly increased; i.e., the suspension stability is decreased. Pathologically, many conditions have been found which also increase the rate, particularly septicemia and pulmonary tuberculosis. One of the probable reasons for the decreased suspension stability is a clumping together of the cells (agglutination), due often to an increase in the globulin and fibrinogen content of the plasma. The ratio of cholesterol to phospholipids also has some influence upon the sedimentation rate. Cholesterol and its esters increase it, while the phospholipids decrease it.

## BLOOD PLASMA

Blood plasma is a light straw-colored fluid having a specific gravity of from 1.015 to 1.035. It is evident that the higher specific gravity of whole blood must be ascribed to the erythrocytes, the specific gravity of which is about 1.090. As previously stated, the specific gravity of plasma is related to the protein content,



and an approximation of the total protein of plasma may be obtained by determining the specific gravity and applying the following formula (Moore and Van Slyke):

$$\text{Percentage of total protein} = (\text{Specific gravity} - 1.007)360$$

Human plasma contains from 90 to 92 per cent water. Blood owes much of its physiological importance to its high water content, for not only is water the medium for carrying the water-soluble and water-dispersible substances present, but it also is needed for maintaining blood pressure, osmotic conditions, and heat regulation. As regards heat regulation, it should be remembered that water has (1) high specific heat, (2) high heat conductivity, and (3) high latent heat of evaporation. Thus water has great heat storage properties; that is, more calories of heat are required to raise the temperature of water a given number of degrees than for most fluids. Its high conductivity results in the rapid removal of heat from the interior of the body by conduction through the water in all the soft tissues and body fluids, as well as in the blood. Finally a great deal of heat is lost through evaporation from skin and lungs, the water coming largely from the blood plasma.

Proteins form most of the solid matter of plasma. They total between 6 and 7 per cent of the plasma. They include fibrinogen, serum albumin, and serum globulin. The globulin and perhaps the albumin fraction each consists of more than one individual protein. Their approximate concentrations in human plasma, as determined by electrophoretic analysis, as well as by chemical analysis, are shown in Table XXIII.

TABLE XXIII

DISTRIBUTION OF HUMAN PLASMA PROTEIN COMPONENTS IN NORMAL ADULTS AS DETERMINED BY ELECTROPHORETIC AND SODIUM SULFATE FRACTIONATION\*

ELECTROPHORETIC FRACTIONATION†			SODIUM SULFATE FRACTIONATION		
COMPONENT	PERCENTAGE		COMPONENT	PERCENTAGE	
	OF TOTAL PROTEIN†	CONCENTRATION GM./100 ML.		OF TOTAL PROTEIN‡	CONCENTRATION GM./100 ML.
Total protein		6.03-6.72	Total protein		6.0 -8.0
Albumin	55	3.32-4.04	Albumin	67	4.3 -5.0
Globulin					
Alpha-1	5	0.31-0.32	Pseudoglobulin II	7	0.2 -0.8
Alpha-2	9	0.48-0.52			
Beta	13	0.78-0.81	Pseudoglobulin I	19	0.8 -1.9
Gamma	11	0.66-0.74	Euglobulin	4	0.1 -0.4
Fibrinogen	7	0.34-0.43	Fibrinogen	3	0.17-0.25
Total	45	2.71-2.72	Total globulin	33	1.9 -3.3

\*From Metcoff, J., and Stare, F. J.: *The Physiologic and Clinical Significance of Plasma Proteins*, New England J. Med. 236: 26, 1947.

†The distribution of components in normal pooled human plasma as derived from electrophoretic analysis is based on the total refractive increment contributed by each component. The quantitative amount of each fraction is based on nitrogen analysis assuming the conventional conversion factor of 6.25. A further assumption tentatively assigns a similar refractive increment per gram of nitrogen to all components. Data derived from several studies. Fractionation in diethylbarbiturate buffer at pH 8.6.

‡Percentage of total protein represents an approximation.

There is also a small amount of prothrombin in plasma and these coagulable proteins, the albumins, globulins, fibrinogen, and prothrombin have been termed "orosins" by Block. In addition, there is some mucoid present. Although we

speak of individual proteins as being present in blood plasma, it is possible that they are really components of one or more protein complexes and in the living organism may not exist as albumins, globulins, etc. That is, they may be "artifacts," substances formed or broken off by the chemical processes of purification.

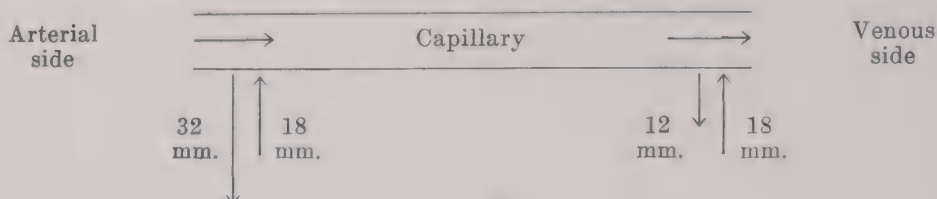
Fibrinogen is the protein which, when blood is shed, is converted into fibrin, the basis of the blood clot. It resembles the globulins in most of its properties but has a few different precipitation reactions. It resembles them in being precipitated by half saturation with ammonium sulfate, but globulins require full saturation with sodium chloride, whereas fibrinogen requires only half saturation. It is formed in the liver. Animals deprived of their liver by surgical operation (hepatectomy) rapidly lose the fibrinogen of their blood. The fibrinogen content of plasma increases when inflammatory or infectious processes exist; also during menstruation and pregnancy. The liver is also the site of formation of the albumin, prothrombin, and probably more than 80 per cent of the globulins. (Post and Patek; Miller.) Normally the plasma proteins are broken down, or catabolized, continuously, but rather slowly, and the rebuilding process, or anabolism, keeps pace with this, so that the level of these proteins remains constant.

### Albumin and Globulin

Albumins and globulins comprise most of the proteins of blood plasma, and it is the relative concentrations of these two proteins, as well as the total protein content, which are of great importance. The reason for this importance is that under normal conditions the colloidal osmotic pressure of the blood, due almost entirely to the proteins of the plasma, is the force which opposes the hydrostatic pressure in the capillaries. The *total* osmotic pressure is the osmotic pressure due to the electrolytes and organic crystalloids present plus that due to the colloids. The electrolytes and organic crystalloids are quite diffusible and pass through the capillary walls into the tissue fluids rather freely. Hence the osmotic pressure which they exert is the same on both sides of the capillary. The plasma proteins, however, are not so freely diffusible and are greater in amount within the capillaries than without. Therefore, the osmotic pressure which they exert, small though it is, is greater inside than outside the capillaries. This is a pressure exerted inward, a suction, and normally just balances the hydrostatic pressure (due to the heartbeat, elasticity of the arteries, etc.) which is exerted outward. This may be made clearer by the following figures and diagram. The total osmotic pressure of plasma is about 6.5 atmospheres or 4940 mm. of mercury. This is a suction and is *almost* balanced by a similar osmotic pressure (suction) of the tissue fluids bathing the capillary. The slight difference is due to the difference in protein concentration between the plasma and tissue fluids.

Osmotic pressure of plasma proteins is about	28 mm. Hg
Osmotic pressure of tissue fluid proteins is about	10 mm. Hg
Difference	18 mm. Hg

Therefore, the *effective* osmotic pressure of the plasma proteins is 18 mm. Hg. The hydrostatic pressure inside and outside the capillaries will vary in different locations and under different conditions, but assuming that the effective hydrostatic pressure is 32 mm. Hg at the arterial side of a given capillary and falls to 12 mm. at the venous side, the following state of affairs would then exist:



As a result, at the arterial side the hydrostatic pressure exceeds the colloidal osmotic pressure ( $32 - 18 = +14$  mm.) and fluid tends to be forced out. At the venous side the reverse is true ( $12 - 18 = -6$  mm.) and fluid is sucked back. Thus filtration is favored at the arterial side and absorption at the venous side. It is easy to see that if the protein content of the plasma were to fall, the effective colloidal osmotic pressure would drop, more water would be forced out, and less would be absorbed. The water of the blood would pass into the tissues and *edema* would result. More than 80 per cent of this colloidal osmotic effect is attributable to the albumin fraction. The reason for this is that there is more albumin, with a comparatively low molecular weight (about 70,000), than globulins, possessing molecular weights over 165,000. Pathologically, damaged kidneys eliminate proteins in about the same relative proportions as they are present in normal plasma. This means a greater loss of albumin than globulin, a particularly unfavorable occurrence if the plasma is already low in albumin. Experimentally, edema may be produced by "plasmapheresis." This procedure consists in repeatedly bleeding the animal and injecting the blood cells after they have been washed and suspended in a protein-free saline solution. When the protein content of the plasma falls below a critical level, edema occurs for the reason given before. There are a number of mechanisms which might lead to a low protein content; e.g., loss of protein via the kidney (in nephrosis, for example), inadequate or improper protein intake, inhibition of plasma protein synthesis and loss of protein as a result of increased permeability of the capillaries.

Edema may result from a number of other causes besides the one described. The principal group of causes refers to general or local changes in capillary blood pressure. That is, if the arterial pressure is relatively lower than the venous pressure, there will be a back pressure and a slowing up of capillary flow with a distention of the capillaries and consequent forcing out of fluid by this increased capillary pressure. This is frequently produced as a result of heart failure or mechanical obstruction of the large veins. Increased permeability of the capillaries may also produce edema. This may be secondary to these changes in capillary pressure or may be brought about by avitaminosis, as in beriberi and scurvy, by bacterial or other toxins, or by extreme heat.

Other functions of the proteins, besides the clotting power of fibrinogen, are their nutritive effects and their buffering power. All of these will be considered in other chapters. Most of the substances concerned in immunological reactions



are of protein nature. At any rate, the *antibodies* which are present in blood, or are produced there, are modified serum globulins. When a foreign protein, an *antigen*, is injected parenterally into an animal, an antibody is formed which is present in the serum of the animal and may be demonstrated by tests *in vitro*. The reactions in such tests are termed "precipitin" reactions if the antigen used is of molecular size. They are called "agglutinin" reactions if the antigen is of cellular size, and "lytic" reactions if the cellular antigen is lysed.

**Regeneration of Plasma Proteins.**—Fibrinogen is produced by the body with remarkable speed. Drury and McMaster bled rabbits and injected the defibrinated blood into the same animals. It was found that after removal of most of the fibrinogen, it was almost completely regenerated in five or six hours. This did not occur if the animals had been hepatectomized. The other plasma proteins are not replaced so quickly, however. Whipple and his co-workers have studied this problem extensively, using plasmapheresis to deplete the organism of plasma proteins. In the first place, if the proteins are reduced to a concentration of between 1 and 2 per cent of the plasma, death usually occurs. If it is reduced to about half the normal value, regeneration is fairly rapid for the first twenty-four hours, during which about one-third of the deficit is restored. Thereafter, regeneration proceeds more slowly until the full quota of protein is restored, in from seven to fourteen days. The diet of the animal plays an important role in plasma protein formation, proteins containing a suitable assortment of amino acids being necessary for rapid regeneration. These studies have shown that the liver is the chief site of formation of these proteins also, although the intestine may have some part in this function. Normally there is a considerable reserve of plasma protein-forming material in the body. This reserve may be reduced by a low protein diet, by fasting, or by plasma depletion (plasmapheresis or hemorrhage). When such depletion occurs, the animal is much less resistant to infection or poisoning. However, such states can be remedied readily by feeding adequate protein together with other suitable nutritive factors.

**Electrophoretic Analysis.**—Electrophoretic studies of blood plasma and serum have increased in number and interest recently and will continue to gain in importance as the results become clarified. The chief difficulty until recently was in the size and cost of the apparatus required, but technical improvements have remedied this considerably. A brief discussion of electrophoresis will be found on page 113. It was pointed out that proteins move in an electric field because of the electric charges which they carry. In the Tiselius apparatus the diluted and dialyzed serum or plasma is overlaid with a buffer solution. During electrophoresis the various proteins at the boundary between the buffer solution and the plasma become separated and move at different rates of speed. As they move, the relative positions and widths of the protein boundaries may either be visibly projected upon a ground-glass screen or recorded on a photographic plate. The albumin fraction moves faster than the globulins or fibrinogen, and a study of the patterns obtained reveal six distinct proteins (Fig. 18). The area of each "hump"

measures the concentration of the protein moving with that particular mobility. Careful study has shown that fractions formerly designated "pseudoglobulin," "euglobulin," etc., which were obtained by salting-out methods, were really mixtures of several globulins. The older terminology has therefore been abandoned and the nomenclature of Tiselius is now generally adopted. The proteins shown to be present are: albumin,  $\alpha_1$ -,  $\alpha_2$ -,  $\beta$ -, and  $\gamma$ -globulins, and fibrinogen. Probably there is more than one albumin present even though this does not appear on the electrophoretic curve. The stationary peaks ( $\delta$ ,  $\epsilon$ ) on the pattern are believed to be boundary anomalies, due to nonprotein factors. It should be mentioned that the patterns will differ with different species, and also, even in the case of the same sample, with the buffer used and with the period of electrophoresis. As seen in Fig. 18, the albumin peak is the tallest and best defined while the globulin and fibrinogen peaks are lower and often spread over a greater distance. The ascending and descending boundaries are not identical.

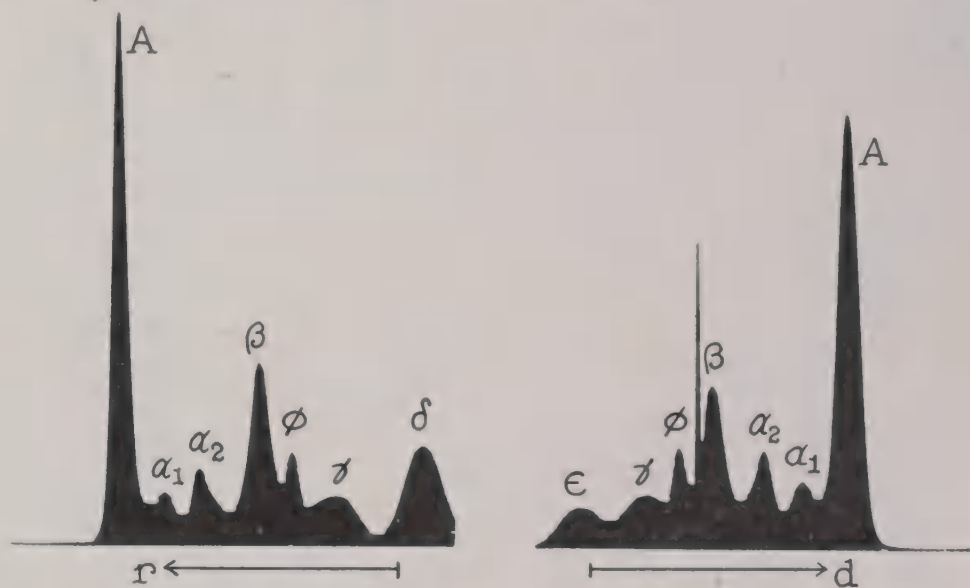


Fig. 18.—Electrophoretic pattern of normal human plasma in 0.1N Na V at pH 8.6. The peaks shown are, from the extreme ends inward, albumin,  $\alpha_1$ -,  $\alpha_2$ -, and  $\beta$ -globulins, fibrinogen,  $\gamma$ -globulin, and the anomalous peak. (From Longworth, L. G.: *Chem. Rev.* 30: 323, 1942; Williams & Wilkins Co.)

Electrophoresis does not separate the protein components into "chemically pure" substances. According to Tiselius, the albumin component contains bilirubin, and the  $\beta$ -globulin, cholesterol. All of the protein fractions contain carbohydrate and some lipid material. During *chemical* separation most of these "combinations" are broken; consequently it is highly probable that the "purified" products thus isolated are really derivatives of the protein complexes as they circulate in the blood, and as they are prepared by electrophoresis.

The functions of the various fractions are beginning to be understood. All take part in the osmotic pressure phenomena previously described, but the albumins, having a smaller size, lower molecular weight, and a higher con-

centration than the others, contribute most to them. The albumins and probably other plasma proteins are concerned in nutritive functions, although they are actually rather poor proteins nutritionally, since they contain little isoleucine and tryptophan. Plasma proteins upon intravenous injection are metabolized and it is presumed that those normally circulating are similarly utilized. Antibodies are for the most part associated with the  $\gamma$ -globulin fraction (Cohn). Among these are the antibodies reacting with diphtheria toxin and those reacting with the viruses of mumps, influenza, and poliomyelitis. The function of fibrinogen in blood-clotting will be discussed in a subsequent section of this chapter. One protein, called "siderophilin," in the  $\beta$ -globulin fraction, carries iron.

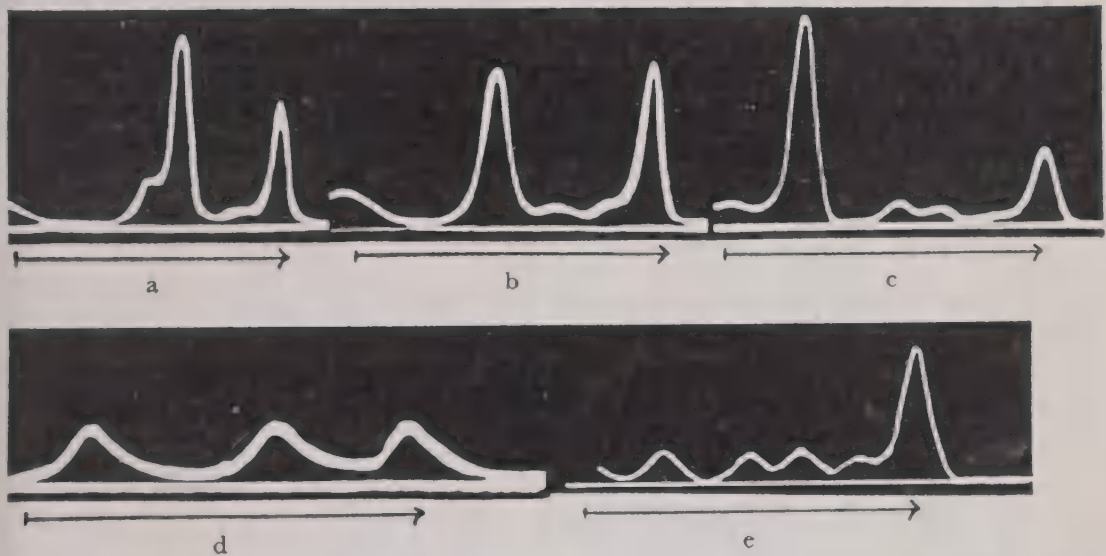


Fig. 19.—Representative electrophoresis diagrams of sera of patients with multiple myeloma. *a*, Alpha type; *b*, beta type; *c*, gamma type; *d*, multiple peaks; *e*, minor anomalies. Ascending boundaries. (From Reiner, M., and Stern, K. G.: *Acta Haematol.* 9: 19, 1953.)

A great variety of studies of pathological plasmas and body fluids have been made, and a few generalizations are permissible. (Stern and Reiner.) In pneumonia, for example, and probably in all febrile conditions, the  $\alpha$ -globulin fraction is increased. In most conditions in which an antigen-antibody system is involved, the  $\gamma$ -globulin fraction is found to be definitely elevated. The blood serum in cirrhosis of the liver is extremely abnormal. (Gray and Barron-Guzman.) The same is true of nephrosis, but the protein-containing urine of nephrotic subjects produces an electrophoretic pattern closely resembling normal serum (Longsworth and MacInnes), indicating that in nephrosis the urinary protein is not entirely albumin as was formerly believed. In multiple myelomatosis, a malignant disease of the bone marrow, a variety of electrophoretic patterns is encountered (see Fig. 19). The reason for this is not known.

**Other Constituents of Plasma.**—Included in the small remaining fraction of plasma solids are amino acids and lower peptides, glucose, lipids, lactic acid,



the ketone bodies, nitrogenous waste products, pigments, inorganic salts, enzymes, vitamins, and hormones. *Glucose* is present in a concentration of approximately 0.1 per cent. It is most probably  $\alpha$ ,  $\beta$  D-glucose. Both the *amino acids* and glucose vary in amount with the state of digestion and nutrition. This is also true of the lipids. In general, they rise after meals, reach a high point, level off, and then fall. Abnormally, all vary to a greater or smaller degree. These fluctuations will be considered in Chapter 22. The *lipids* of plasma are fats, fatty acids, phospholipids, cholesterol, and cholesterol esters. The phospholipids include lecithin, a smaller amount of sphingomyelin, and a very little cephalin. Most of the lipid is bound as the  $\beta$ -lipoprotein, which represents about 5 per cent of the normal plasma proteins. (Oncley, Gurd, and Melin.) *Lactic acid* is present normally in small amounts and increases with exercise, and also under pathological conditions. It is a product of carbohydrate metabolism. Traces of the *ketone bodies*, namely, acetone, diacetic acid, and beta-hydroxybutyric acid, also are normally present. They are derived from fatty acids and are increased when there is interference with fat metabolism. The *nitrogenous waste products* result from the breakdown of proteins, purines, and other nitrogen-containing substances present in food and tissues. Included are urea, uric acid, creatinine, hippuric acid, and others. Their presence in blood is an indication of the transportation of waste products by the blood stream to the kidneys and other organs of elimination. Creatine is also present in minute amounts. The pigments of normal plasma probably include the urinary pigments, bile pigments, and carotene, all in traces, but abnormally these may be increased and others added.

The positive *inorganic* ions present in blood in appreciable amounts are  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$ . The negative are  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^-$  and  $\text{HPO}_4^-$  (see Fig. 54). In addition, lactic and other organic acids and proteins contribute somewhat to the ionic picture. In plasma and, in fact, in all body fluids, the concentration of sodium ions exceeds that of potassium; in the blood cells, and other cells, the reverse is true. The bicarbonates and the phosphates are quite important as buffers and the shifting of the chloride ion in and out of the erythrocytes has a definite role in acid-base balance. Changes in the concentrations of many of these ions occur under pathological conditions; for example, the  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations fall and that of  $\text{K}^+$  rises in Addison's disease, and  $\text{Ca}^{++}$  is diminished when parathyroid function fails. This is also the case as regards some of the elements and ions present in traces; e.g., iodine and iodides, sulfo-cyanates, copper, and iron.

There are an amylase, an invertase, and glycolytic enzymes present in blood plasma. The glycolytic enzymes are very important from a practical standpoint. When a blood sample is taken from an individual, the concentration of glucose will be found to diminish slowly as a result of the action of these enzymes. Consequently, sugar analyses should be begun as soon as possible after the blood is obtained. Other enzymes present are catalase, lipase, protease, phosphatases, peptidase,  $\beta$ -glucuronidase and choline esterase.

## RED BLOOD CELLS

The red blood cells, or erythrocytes, are formed in the bone marrow. The control of the production of these cells depends upon a hematopoietic substance, or erythrocyte-maturation factor. The mechanism of its production will be described in greater detail later. However, in addition to this factor, which is stored in the liver and stimulates maturation of the red blood cells in bone marrow, there are required for normal erythropoiesis suitable and adequate dietary protein, "available" iron salts, and traces of copper and cobalt.

The red cells contain less water than the cells of most tissues; namely, about 60 per cent. Most of the solid matter is hemoglobin, the conjugated protein which is the red coloring matter of blood. The stroma, or meshwork, is composed of other proteins and lipids, to which the hemoglobin is probably bound intimately. The red cell is thought to have a membrane, an extremely delicate covering composed, perhaps, of the same substances as are present in the rest of the cell but in greater concentration. The lipids are chiefly cholesterol, lecithin, and cephalin, and the proteins include an albuminoid, "stromatin," and a lipoprotein, "elinin." Elinin seems to possess antigenic properties (Calvin and others). Another protein of the stroma is hemocuprein, a bluish copper-containing substance, the function of which is unknown. Various enzymes are present, including carbonic anhydrase, catalase, peptidases, choline esterase, and the enzymes of the glycolytic system (see page 423). All of the glutathione of blood is located in the red cells. Adenosine di- and triphosphates (see page 422) and di- and triphosphopyridine nucleotides (see page 347) are also important constituents of the erythrocytes. Soluble organic crystalloids present include urea, amino acids, creatinine, and glucose. The concentration of glucose is about the same in the red cell as in plasma. The electrolyte composition of the red cells is qualitatively similar to that of the plasma. It differs quantitatively, however. There is more potassium than sodium—just the reverse of the relationship of these two elements in plasma. The osmotic pressure of the interior of the red cell is equal to that of the plasma. (This is normally equivalent to the osmotic pressure of a 0.9 per cent NaCl solution, which is termed "normal" or, better, "physiological" saline, since it is not the same as a chemically "normal" solution.) Changes in osmotic pressure of the medium surrounding red blood cells influence their size. If the solution is hypotonic, water will pass into the cell and its size will increase. Not a very great increase occurs before the cell bursts and the hemoglobin is released. This process is called hemolysis or laking, and such blood, which is a clear transparent crimson fluid, is laked or hemolyzed blood. By putting the red cells in a hypertonic solution, i.e., one which has a higher osmotic pressure than 0.9 per cent NaCl, the cells shrink and take on a shrivelled appearance. These are described as "crenated" cells. Hemolysis may be produced by other means besides the one mentioned. Substances which dissolve or change the physical state of the lipids, such as ether, chloroform, bile salts, and soaps, will accomplish the same purpose. Certain biological toxins, especially those produced by venomous snakes and hemolytic bacteria, also cause the laking of red cells. Some contain en-

zymes which hydrolyze lecithin and others act by solution of, or combination with, lipids. Physical forces, such as irradiation with ultraviolet rays, and alternate freezing and thawing, may so alter the structure of the cell as to cause the release of hemoglobin. Aging, also, has a similar effect, and this is why whole citrated blood, kept in blood banks, cannot be used after from five to seven days. The red cells become more and more fragile. The addition of glucose prolongs the serviceable period to from sixteen to thirty days, under proper conditions. Hemolysis may occur in the human body under pathological conditions, but it never occurs in the body as a result of lowering osmotic pressure. When it does occur, as a result of the action of bacteria, venoms, or other agents, the hemoglobin released into circulation is excreted by the kidney, resulting in hemoglobinuria.

### Hemoglobin

Hemoglobin is a conjugated protein, with a prosthetic group, heme, united to the protein, globin. The pigmentary property and chief respiratory functions are associated with heme, the iron-containing pigment, but it must be mentioned

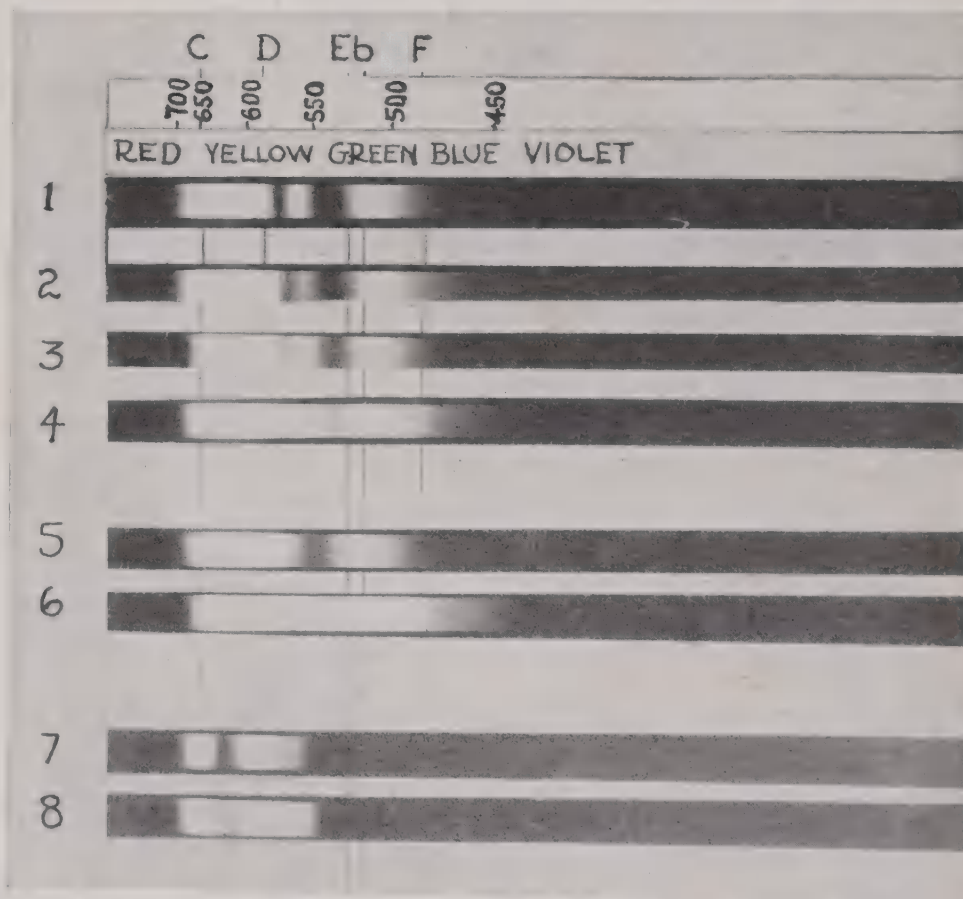


Fig. 20.—Absorption spectra. At the top are shown the positions of the reference lines, next is a millimicron scale, below which are indicated the positions of the colors of the solar spectrum. The spectra below must be pictured as having these same spectral colors wherever light appears, the dark portions appearing black because of absorption of the light rays.

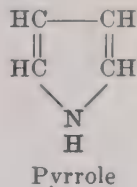
1, Oxyhemoglobin; 2, 3, and 4, carbon monoxide hemoglobin of different concentrations (compare with 1 and note the slight but definite difference in the position of the two bands and also the disappearance of one absorption band in the most dilute solution, 4); 5 and 6, hemoglobin of different concentrations; 7 and 8, methemoglobin of different concentrations.



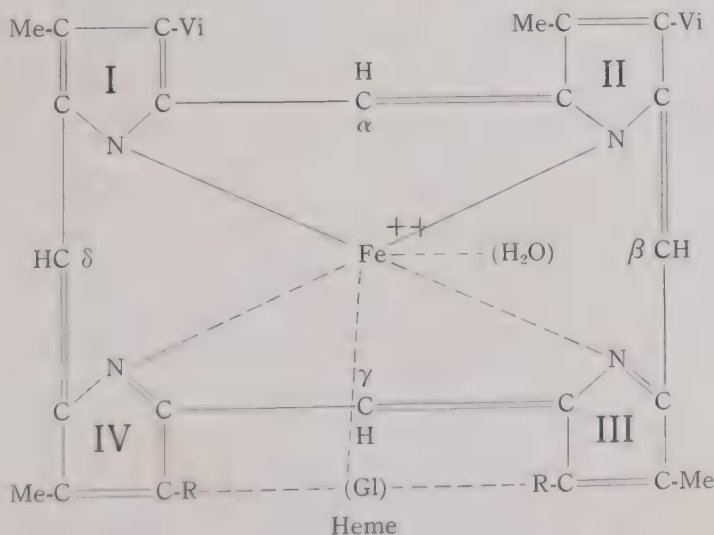
that the globin fraction plays a role in carrying  $\text{CO}_2$  (see page 537). It is a crystallizable protein and, according to Reichert, each species has its own peculiar crystalline hemoglobin. It has the power of uniting in loose combination with atmospheric oxygen, forming oxyhemoglobin. This occurs in the capillaries surrounding the alveoli of the lungs, and the oxygen is thus transported in the arterial blood to the tissues, where part of it is released, and the venous blood, somewhat depleted of its oxygen supply, returns to the lungs for oxygenation.

Hemoglobin, its derivatives, and other related compounds have characteristic absorption spectra. That is, if such a solution is interposed between a source of white light and the prism of a spectroscope, the light of certain wave lengths is absorbed, and dark bands, or shadows, appear in the spectrum wherever the light has been taken out. Thus, hemoglobin, when in the deoxygenated state ("reduced hemoglobin"), has one broad band in the yellow-green section, its center being at  $559 \text{ m}\mu$ . Oxyhemoglobin has two narrow bands. One, the narrower of the two, is in the yellow, with its center at  $579 \text{ m}\mu$ . The wider one is nearer the green, with its center at  $542 \text{ m}\mu$ . On dilution, the wider one disappears first. Extremely dilute solutions of hemoglobin may be detected spectroscopically. This method has various practical applications in the recognition of a number of derivatives of hemoglobin. In Fig. 20 is shown the absorption spectra of some of these compounds.

**Chemical Structure of Heme.**—Heme is an iron porphyrin, a porphyrin being a union of four pyrrole groups.

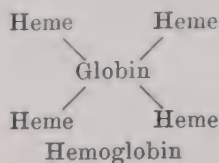


Heme may be represented by the following structural formula:



where Me = methyl, Vi = vinyl, Gl = globin, and R = propionic acid

It should be noted that the arrangement of the double bonds varies in the different pyrrole groups. Free heme does not contain globin or  $\text{H}_2\text{O}$ , but hemoglobin does. Four molecules of heme unite with one of globin to form hemoglobin. It may be represented by the following scheme:



For each heme group, one of the coordination valences of the iron is believed to be connected to one of the imidazole N's of a histidine component of globin. The other two linkages to globin are postulated to be combined with the two propionic acid groups present in heme.

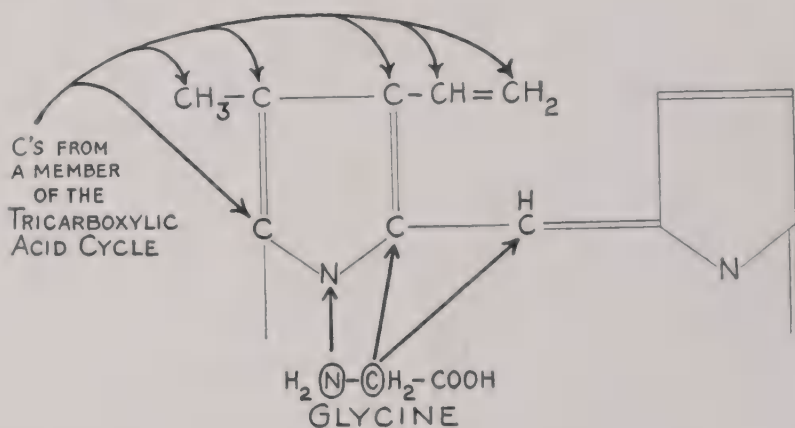


Fig. 21.—Diagram of part of heme molecule indicating sources of nitrogen and carbon atoms.

The alpha carbon of glycine is shown as the source of the methene bridge carbon as well as of one pyrrole carbon, the exact position of which in the ring is not known.

Our knowledge of the synthesis of heme in the body has been extended very materially by the use of isotopically labeled compounds. Not only can the animal as a whole synthesize the pyrrole unit, but it has been demonstrated that heme can be synthesized *in vitro* by mammalian reticulocytes and by the nucleated red blood cell of birds. Rittenberg, Shemin, Altman, Kamen, London and their co-workers have demonstrated these phenomena and have determined the sources of the individual parts of heme in several brilliant series of experiments. (See Fig. 21.) The initial findings demonstrated that glycine and acetic acid are concerned with the atoms of the porphyrin. The nitrogen of glycine is utilized for both types of pyrrole rings; that is, those which have vinyl groups and those which have propionic groups. The carboxyl carbon of glycine is not used for heme formation, but the  $\alpha$ -carbon is. Of eight such  $\alpha$ -carbons of glycine which go into each porphyrin molecule, four are the source of the methene bridge carbon atoms, and four others go, one each, into comparable positions of each pyrrole unit. The remaining carbons are derived from succinic acid, a member of the tricarboxylic acid cycle. (See page 426.)

Since acetic acid enters into metabolism by way of the tricarboxylic acid cycle, it is evident that this is the pathway by which the two-carbon acid is introduced into the porphyrin structure. (Shemin and Wittenberg.)

Tracer nitrogen was also used to study the life span of red cells. After the feeding of labeled glycine was stopped, the concentration of labeled heme did not level off and decrease as would be expected if the red cells were rapidly being catabolized. It continued to increase for nearly 25 days and then leveled off until about the seventieth day. At this time the  $N^{15}$  content began to diminish. The life span of the average red cell was found to be about 85 days for the dog and 120 days and 109 days for the adult man and woman, respectively (Grinstein; London). It is quite possible that pteroylglutamic acid (see page 300) has an influence upon the biosynthesis of porphyrins. (Totter.)



Fig. 22.—Hemin crystals from human blood.

The fact that glycine, an “unessential” amino acid, is required for the formation of heme indicates that it is really essential from this standpoint. Indeed some nutritionists believe that those amino acids which are called “unessential” are so important for vital functions that the body has “learned” to synthesize them.

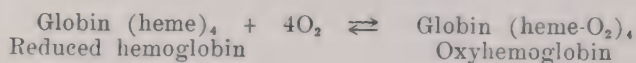
The iron in reduced hemoglobin is in the ferrous state. In oxyhemoglobin it is still in the ferrous state, but there is more oxygen present. This is assumed to be linked loosely to the Fe by a residual valence force. Other porphyrins exist in nature or can be synthesized; some have variations in the side chains, and others may have some heavy metal, other than iron, or no metal at all. A



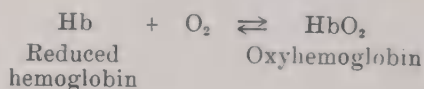
*hemochromogen* is a compound of heme with any nitrogenous substance. Hence, hemoglobin and most of its derivatives are hemochromogens, as are also the respiratory pigments of the invertebrates, as well as the cytochromes, substances which effect oxidations in the tissues.

Heme may be oxidized to "hematin," which contains an -OH radical. This -OH may be substituted by -Cl, forming "hemin." Hemin crystallizes out in characteristic brown crystals, which may be easily recognized under the microscope. (See Fig. 22.) This procedure is used as a test for blood. A droplet of blood is placed on a microscope slide and warmed under suitable conditions with acetic acid and sodium chloride. This splits off the heme from the hemoglobin, oxidizes it, and permits the reaction with -Cl.

Since each molecule of hemoglobin has four heme groups, it contains four  $\text{Fe}^{++}$  atoms. Each heme radical unites with one molecule of oxygen,  $\text{O}_2$ . We may accordingly represent reduced hemoglobin as globin (heme)<sub>4</sub> and the reaction whereby it performs its respiratory function as:



Usually, however, in discussions concerning this reaction a less exact expression is used, namely:



Most of the oxygen present in arterial blood is held in this loose chemical combination with hemoglobin. The ease with which this union may be brought about and broken may be demonstrated in the laboratory. Blood, which has been rendered nonclotting, may be poured from one vessel to another a few times and it becomes bright crimson (oxyhemoglobin). Addition of a mild reducing agent changes its color to a very dark red (reduced hemoglobin), after which it may be oxygenated again as before. These changes may be followed spectroscopically if the blood is suitably diluted. In addition to the oxygen combined with hemoglobin there is a small amount held in solution in the plasma. The tension, or pressure, of oxygen in the plasma, together with other factors, determines the rapidity and degree of dissociation of oxyhemoglobin into oxygen and hemoglobin. The partial pressure of the oxygen in atmospheric air at the barometric pressure of 760 mm. Hg is 159 mm. Hg; i.e., 20.9 per cent  $\text{O}_2 \times 760$  mm. Hg. If blood is placed in contact with oxygen at this pressure, the hemoglobin becomes completely converted to oxyhemoglobin. Increase of oxygen pressure can add no more oxygen to the hemoglobin, but can force more oxygen into solution in the plasma. Lowering the partial pressure of oxygen causes dissociation to occur, but even at 102 mm. Hg, which is the partial pressure of oxygen in arterial blood, the hemoglobin is 95 per cent saturated. Still lower pressures, such as obtain in the tissues, cause further dissociation or release of atmospheric oxygen near the site of tissue oxidations. This discussion will be continued when the chemistry of respiration is taken up (Chapter 20).

**CARBON MONOXIDE HEMOGLOBIN.**—Carbon monoxide combines with the heme portion of hemoglobin to form carbon monoxide hemoglobin, called also carboxy-hemoglobin and carbonyl hemoglobin. This is a much firmer combination than the one between oxygen and hemoglobin. The affinity of hemoglobin for CO is about 210 times that for O<sub>2</sub>. If, therefore, carbon monoxide is in the inspired air, it will form this firm combination to a greater extent than its proportion in the air would seem to warrant. Consequently, if enough CO is present, the blood will not have sufficient oxyhemoglobin for respiratory purposes and asphyxiation will occur. Carbon monoxide hemoglobin has a cherry red color which is not changed readily by reducing agents. Its absorption spectrum resembles that of oxyhemoglobin, but the two bands are slightly nearer the violet end of the spectrum. Their centers are at 570 and 535 m $\mu$ , respectively. Carbon monoxide hemoglobin may also be detected by chemical tests. The simplest is to dilute the suspected blood greatly, after treating it with a little NaOH, and compare the color with normal blood similarly handled. Normal blood shows a greenish hue after such treatment, whereas CO blood remains pink.

Poisoning by carbon monoxide is the most common form of poisoning in modern life. Carbon monoxide is particularly dangerous for two reasons: First, it is odorless and colorless, and consequently cannot be readily detected; second, its action is insidious and rapid. The victims frequently become unconscious in a few minutes and death often follows quickly.

This gas is found wherever incomplete combustion of carbonaceous materials occurs—in automobile exhaust gas (4 to 7 per cent), in chimney gases and smoke, and in blasting gases. It is also a constituent of illuminating gas, in which its percentage varies from 4 to 40 per cent, depending upon the source materials and the method of manufacture. Poisoning may be either of an acute or a chronic nature. Both are important from the standpoint of public health. Deaths resulting from the inhalation of automobile exhaust gas have increased alarmingly in the past few years. An automobile emits in its exhaust 1 cubic foot of CO per minute per 20 horsepower. In a small individual garage with no ventilation, this amount may be fatal to a person in five minutes. It is therefore imperative that a door or window of a garage always be open, even in the coldest weather, when the motor is running. It should also be apparent that vehicular tunnels are hazardous because of the possible accumulation of CO from automobile exhaust gas in the atmosphere. The adequate ventilation of such tunnels is consequently of utmost importance.

There are several factors which determine the degree of toxicity of CO, but all relate to one point; i.e., the rate of absorption of this gas. The chief factors are (1) concentration of CO in the air respired, (2) duration of exposure, and (3) rapidity of respiration. The rate of respiration depends upon the activity of the individual, his age and size, and the temperature and humidity of the atmosphere. The symptoms produced will depend upon the percentage of hemoglobin combined with carbon monoxide and thus rendered physiologically useless, at least for the time being. If the percentage of hemoglobin saturated with CO is

- 0 to 10 per cent, no symptoms usually are seen;
- 10 to 20 per cent, there is possibly a slight headache;
- 20 to 30 per cent, headache, throbbing in temples;

- 30 to 40 per cent, severe headache, weakness and dizziness, dim vision, nausea, vomiting, possibly collapse;  
 40 to 50 per cent, like the above but with greater possibility of collapse, increased pulse and respiration;  
 50 to 60 per cent, unconsciousness, coma with intermittent convulsions, Cheyne-Stoke's respiration (a rhythmic, periodic type of respiration);  
 60 to 70 per cent, like the above, but with depressed heart action and respiration, possibly death;  
 70 to 80 per cent, weak pulse, respiratory failure, death.

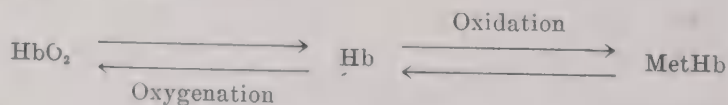
The proportion of CO in the air necessary to produce such CO saturation figures will depend upon the factors first mentioned, but in a general way it may be said that if the respired air contains

- 0.01 per cent, symptoms begin after a few hours;  
 0.04 per cent, it is safe for only about one hour;  
 0.10 per cent, it is uncomfortable and may be dangerous in two hours;  
 0.30 per cent, it is dangerous in thirty minutes;  
 0.60 per cent, it is dangerous in ten to fifteen minutes;  
 1.30 per cent, it is dangerous in one to three minutes.

The treatment in cases of CO poisoning is (1) rapid removal from the poisoned atmosphere, (2) artificial respiration, using an O<sub>2</sub> and CO<sub>2</sub> mixture if available, and (3) blood transfusion, if necessary.

Chronic carbon monoxide poisoning may result from a leakage of CO into the respired air in a number of ways. Defective furnaces, leaky gas pipes or fixtures, and smoke from chimneys are examples. Workers in railroad roundhouses or in garages are also subject to this condition for obvious reasons. The symptoms are too diverse to be enumerated here. It is said that they may resemble a number of common illnesses or conditions, e.g., colds, rheumatism, dietary indiscretions, and hysteria, and thus delay diagnosis. The symptoms most frequently mentioned, however, are "tightness" across the forehead, headache, flushed face, cherry red patches on the skin, noises in the ears, weakness, impairment of vision, dizziness, dyspnea, nausea, and vomiting. Even slightly gassed persons require careful attention. In the zeal to provide them with fresh air, care should be taken to avoid *cold* air.

**METHEMOGLOBIN.**—Methemoglobin is a derivative in which the iron is in the ferric state. It is produced by the oxidation of hemoglobin, as, for example, when potassium ferricyanide is added to blood. This is quite a different thing from *oxygenated* hemoglobin; i.e., oxyhemoglobin. In the latter the oxygen is united loosely with *ferrous* iron. In methemoglobin the iron is oxidized to the *ferric* condition, and, in fact, oxygen is liberated in this reaction, leaving the methemoglobin devoid of this gas. It should be noted that while hemoglobin may be oxidized to methemoglobin, oxyhemoglobin cannot be. Thus the following relationship may exist:



A small amount of methemoglobin develops very slowly in shed blood. Its reduction to hemoglobin also occurs spontaneously and this seems to be bound up with glycolytic reactions (see page 174) in the erythrocyte (Drabkin). After



the administration of certain drugs or exposure to certain poisons, methemoglobin is likely to be present in circulating blood. These include chlorates, acetanilide, nitrites, nitrobenzene, antipyrine, iodine, phenacetin, sulfonal, trional, and, perhaps most important today, the sulfonamide drugs. Moreover, methemoglobin occurs in considerable amounts in the blood of certain individuals as a familial disease or inborn error of metabolism. In such cases, the methemoglobin is found only in the red cells, while methemoglobin resulting from poisoning is found in the plasma. Although methemoglobin is an oxidized substance, it does not carry oxygen as oxyhemoglobin does; hence hemoglobin, which has been changed to methemoglobin, is unable to function as a respiratory pigment. However, it slowly changes over to hemoglobin in the body.

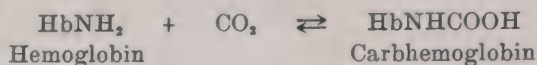
In some industries, poisons which cause methemoglobinemia are produced. Nitrobenzene is used in the manufacture of shoe dyes, floor polishes, cosmetics, and explosives. Workers in these industries may be acutely or chronically poisoned if nitrobenzene is absorbed in sufficient amounts, and in such cases methemoglobin is found to be present in the blood. The fumes from carbon arcs contain NO which reacts with atmospheric oxygen to form NO<sub>2</sub>. If breathed in high concentrations, methemoglobin may be produced. Moving picture operators are constantly exposed to this hazard, but it is believed to be of little danger because the projection booths are usually ventilated adequately. One of the methods of combating this state is to inject glucose or methylene blue intravenously, which helps to reduce methemoglobin (Fe<sup>+++</sup>) to hemoglobin (Fe<sup>++</sup>), the pigment thus becoming again available for oxygen transport. Another procedure is the administration of ascorbic acid, which also has a marked reducing action (Carnrick). Methemoglobin, in alkaline solution, has an absorption spectrum quite similar to that of oxyhemoglobin, but in acid solution there is a characteristic band, toward the red end of the spectrum, with its center at about 634 mμ.

**Other Derivatives of Hemoglobin.**—Hydrogen sulfide reacts with hemoglobin to give a compound having a characteristic absorption spectrum.

HCN and cyanides, however, do not react directly with hemoglobin but do react with methemoglobin to form cyanmethemoglobin. The principal toxic action of the cyanides lies in their combination with cytochrome oxidase. (See page 350.) Therefore the treatment of cyanide poisoning is based on the production of methemoglobin, in order to remove the cyanide from this important enzyme. Sodium nitrite and sodium thiosulfate are injected intravenously. The former induces the production of methemoglobin, which quickly combines with the cyanide. Methemoglobin and cyanmethemoglobin, although not useful respiratory pigments, are not in themselves toxic. Cyanmethemoglobin is slowly converted to hemoglobin and cyanate, which is non-toxic. The sodium thiosulfate also reacts with cyanide, yielding thiocyanate, an innocuous salt, which is readily excreted.

A different type of combination is that of hemoglobin with CO<sub>2</sub> to form carbhemoglobin, a carbamino compound. In this case the combination is with

the globin rather than with the heme. An  $\text{NH}_2$  group is responsible in part, at least:



This is a normal and constant physiological reaction and accounts for from 2 to 10 per cent of the  $\text{CO}_2$  transported by the blood.

## WHITE BLOOD CELLS

The white blood cells, or leucocytes, are much fewer in number than the red cells, and they have a lower specific gravity. Consequently, when whole blood is centrifuged they form a whitish layer above the red cells. Normally there are from 5000 to 10,000 per cubic millimeter. The different types and variations in number cannot be considered here. Suffice it to say that in leucemias and in many infections and inflammatory conditions they are greatly increased in number, while in typhoid fever and in some other abnormal states a leucopenia, i.e., a decreased number of white cells, develops. Since they are typical cells, they contain water, nucleoproteins, albumin, globulin, and other proteins, lipids (especially cholesterol and phospholipids as well as fat), glucose, and other soluble organic substances and inorganic salts. They also possess a great variety of enzymes, and, undoubtedly, hormones and vitamins.

## PLATELETS

The blood platelets are also called thrombocytes and number about 200,000 to 400,000 per cubic millimeter normally. Their numbers increase after hemorrhage and decrease in some types of purpura (purple patches in the skin due to subcutaneous extravasation of blood). The origin of platelets is not well understood. Most authorities incline to the view that they are fragments of protoplasm broken off from the megakaryocytes (giant cells) of the bone marrow. Others claim that they are pieces of damaged red cells and sometimes contain hemoglobin, although ordinarily they are colorless. The exact composition of platelets has not been determined, but they contain a considerable amount of phospholipid, most of it cephalin. There may also be protein and an enzyme present. On disintegration these substances are freed and one or more of them is involved in blood clotting. They agglutinate very readily and also have a vasoconstrictor action. Thus, when small vessels are injured, the agglutinated platelets tend to seal the leaking vessels and prevent further bleeding by vasoconstriction and by aiding clotting if the blood is actually shed.

## BLOOD COAGULATION

The ultimate reaction in blood coagulation is the conversion of the soluble protein fibrinogen, present in colloidal solution, to the insoluble fibrin. This reaction, of course, does not occur normally in circulating blood, although most of the factors are present. It is also maintained that blood will not clot in a section of a blood vessel if the section is carefully tied off and re-

moved from the body. This indicates that the motion of the blood is not the cause of its continuing fluidity. This experiment, however, is only successful if the ligatures do not crush the vessel wall and thus prevent seepage of tissue juice into the ligated vessel segment. The addition of tissue juice induces intravascular coagulation.

As mentioned previously, the coagulation may be prevented if ionized calcium is removed from blood, and the coagulating power is restored when these ions are again added in sufficient amount. The transformation of fibrinogen to fibrin is catalyzed by an enzyme called thrombin. Thrombin has its origin in prothrombin, from which it is derived by a complex activation process, involving the participation of a number of activators, including  $\text{Ca}^{++}$ , found in the plasma, fixed tissues, and platelets. The activation of prothrombin may be inhibited by substances found in plasma and the fixed tissues. The enzyme thrombin survives for only a short time and is then inactivated by antithrombin. (See Fig. 23.)

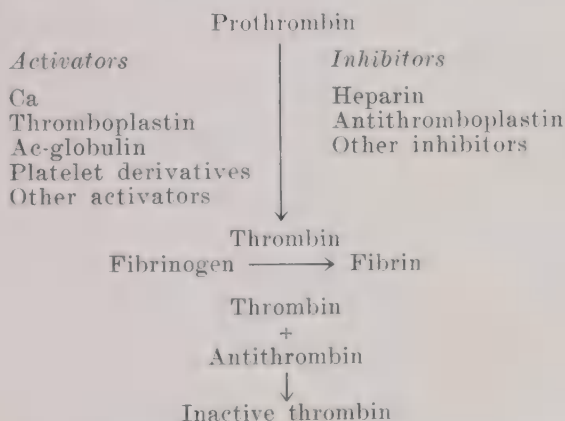


Fig. 23.—Outline of blood-coagulating mechanism.

The chemistry of blood coagulation may thus be discussed in four major categories; namely, (1) the interaction of thrombin and fibrinogen, (2) prothrombin and its activation, (3) the inhibition of prothrombin activation, and (4) the neutralization of thrombin activity. Many procedures for activating, inactivating, and reactivating different parts of the coagulation mechanism have been devised, and the interpretation of these results has been the basis for theories and revisions of theories. The discovery of new factors has led to new names and, since some of these factors proved to be identical, there has resulted a confusion which is difficult to resolve. Many competent investigators have worked in this field, among them Howell, Morawitz, Wöhlisch, Nolf, Copley, Jaques, Smith, Quick, Ferguson, Astrup, Owren, Seegers, Ware, Tocantins, Bordet, and Chargaff. The discussion below presents some of their conclusions, which represent one of the most fascinating perspectives in protein interactions.

**Fibrin Formation.**—In the interaction of thrombin and fibrinogen the main alterations are with fibrinogen. It has a molecular weight of about 340,000, an isoelectric point of pII 5.5, contains practically all the known



amino acids, and is normally found in plasma to the extent of 350 mg. per cent. In the presence of thrombin a fibrinopeptide of low molecular weight arises by cleavage of fibrinogen (Laki; Seegers).



This leaves a protein with uneven electric charge distribution and of different sign on its architectural structure. These electrical charges function to align the molecules laterally and, end to end, to form the fibrin gel. The rate of the above reaction is inversely proportional to the thrombin concentration. The fibrin so formed in laboratory experiments happens to be soluble in concentrated urea solution, whereas the fibrin of a natural blood clot is not. To change the urea-soluble clot to a urea-insoluble clot, calcium ions and a plasma globulin, called the fibrin stabilizing factor, are necessary. How these two substances function to produce such alterations in solubility is not known. The influence of calcium ions is also manifested by an increase in the rate of thrombin-fibrinogen interaction. This rate is also augmented by platelets, and the latter also play an important role in the phenomenon of clot retraction which is associated with the properties of fibrin.

Returning for a moment to the interaction of purified fibrinogen and purified thrombin in laboratory experiments, it is interesting to note that relatively large amounts of thrombin not only form a fibrin gel but in time the gel again dissolves. Fibrin may thus, under proper conditions, be a mere transition state of more extensive changes associated with thrombin. This thus represents thrombin as a special kind of proteolytic enzyme, and from that viewpoint it has been studied in connection with synthetic substrates. Furthermore, thrombin has been shown to produce changes in Ac-globulin, prothrombin, and platelets and can interact with a particular plasma protein to lose its own thrombin activity. This latter reaction is the antithrombin reaction and will be considered subsequently in greater detail as one of the major categories of the subject of blood coagulation.

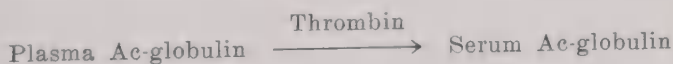
**Activation of Prothrombin.**—Prothrombin is present in blood plasma, provided there is an adequate supply of vitamin K available for its synthesis by the liver. Prothrombin will not enable clotting to occur until changed into thrombin. Purified prothrombin was obtained by Seegers and associates, and their studies with purified materials have helped clarify many difficult uncertainties about this substance. Its normal concentration in plasma is from 10 to 15 mg. per 100 ml. Purified material has a molecular weight of 62,700 and has the physical properties of an albumin, but the amino acid composition is wholly different from that of albumins. Its isoelectric point is near pH 4.2, at which point it is quite insoluble in water. The molecule is extremely sensitive to acids and alkalis and is unstable in solution. In 25 per cent sodium citrate solution, the molecule changes to thrombin autocatalytically. In addition to thrombin, at least two other degradation products are found, including one rich in carbohydrate. Besides being an interesting observation in protein chemistry, the

autocatalytic activation of prothrombin established the fundamental fact that prothrombin in itself possesses the necessary materials from which thrombin may be obtained. This basic concept serves as a guide in considering the many activators of prothrombin found in nature. Some of these may now be considered, even though their chemical action is not known. It is known only that some of them are essential in the normal physiology of hemostasis and that certain combinations of these activators produce thrombin rapidly, while other combinations enable prothrombin to activate only slowly. The "old" diagrams of blood coagulation theories commonly found are now known to be wholly inadequate, even though the chemical work has not advanced far enough for correct representations. It seems safe to predict that no one diagram or proposed mechanism can encompass the vast fund of knowledge which is so rapidly accumulating about these substances.

**Calcium.**—Calcium ions are required, and normal plasma contains about 10 mg. per cent of calcium, about half of which is ionized. This is approximately the optimal concentration for coagulation. Even in severe tetany with extremely low blood-calcium concentrations the calcium level is sufficiently high for clotting. Thus the body always has sufficient calcium present for the formation of thrombin, and attempts to improve clotting conditions therapeutically by raising the blood calcium level have been of little value.

**Thromboplastin.**—This is a term applied to activators of prothrombin derived from fixed tissues such as muscle, lung, and brain. In brain the active material becomes concentrated in crude cephalin fractions, and in subfractions it is associated with phosphatidylserine. In preparations from saline extracts of ground lung tissue the activity resides in a complex protein, perhaps most appropriately referred to as a large lipoprotein (Chargaff). Together with calcium alone these substances do not activate purified prothrombin rapidly.

**Ac-Globulin.**—This protein is found in plasma in trace quantities and plays an important role in the activation of prothrombin. Although powerful concentrates have been prepared in the laboratory, it has not been obtained in sufficient purity for chemical study. A small amount of thrombin greatly changes the properties of Ac-globulin so that it becomes an accelerator of prothrombin activation (Ware, 1947). This change has been represented as follows:



The nature of the change of plasma Ac-globulin to serum Ac-globulin is not known. It is, however, clear that an excess of thrombin is associated with the change and ultimate disappearance of serum Ac-globulin activity. In human serum, Ac-globulin is stable for only a matter of minutes, so that serum is practically devoid of Ac-globulin activity. (Murphy and Seegers.) In oxalated human plasma stored as commonly done in a blood bank, plasma Ac-globulin is not stable. With citrate as anticoagulant the activity remains for a few days. The fundamental reason for the greater stability in citrated plasma as compared with oxalated plasma is not known. It could be on the



basis of oxidation mechanisms, since oxygen bubbled through plasma samples tends to destroy Ac-globulin activity. When Ac-globulin is absent from the blood there is a bleeding tendency and the patient is said to have parahemophilia in accordance with the suggestion of Owren, who first described and studied the disease in detail.

**Hemophilia Factor of Plasma.**—The importance of this substance in the normal activation of prothrombin is plainly displayed in some persons in whom clotting does not occur at the normal rate. The condition is called hemophilia and the sufferers from it are termed "hemophiliacs" or, commonly, "bleeders." Hemophiliacs must be extremely careful not to experience even very minor wounds and injuries since these may result in severe and even fatal hemorrhages. The cause of the incoagulability of the blood in hemophilia is not known. Apparently all the clotting factors are present, but there is a delay in the formation of thrombin. One explanation is that the equilibrium of coagulant and anticoagulant substances, or their release into circulation, is disturbed. There may be still some other component of plasma lacking which has not yet been identified. In favor of this explanation is the fact that normal human plasma globulin, when added to hemophilic blood or when injected intravenously, brings about normal coagulation. The assumption is that this unidentified factor is associated with the globulins. Consequently transfusion of blood from a normal individual into a hemophiliac is sometimes indicated. Another hypothesis is based on the work of Tocantins. He has demonstrated that thromboplastin is destroyed or inactivated when incubated with normal plasma and that this inactivation is much more marked if hemophilic blood plasma is employed. If this is the case, thromboplastin is destroyed rapidly in hemophilia and not enough can accumulate to enable coagulation to occur.

In work concerned with the activation of purified prothrombin it has been shown that the plasma globulin, studied by many, can be prepared in concentrated form and, together with platelets and calcium, it activates prothrombin rapidly. This globulin is apparently also in hemophilia plasma but is probably masked by the antithromboplastin substance of Tocantins. Ether extraction of hemophilia plasma makes its capacity to activate purified prothrombin equivalent to that of normal plasma.

**Plasma Thromboplastin Component.**—Patients with a deficiency of this plasma factor have a bleeding tendency quite like classical hemophilia; however, when their blood is mixed with that of the hemophiliac, they mutually correct the clotting defects. This component of plasma can be adsorbed on substances like  $\text{BaSO}_4$ . Its mechanism of action in the activation of prothrombin is not known. The indications are that it is connected with platelet activity.

**Other Activators of Prothrombin.**—Another bleeding tendency has been described. It also is said to be associated with lack of an important plasma protein. This substance has been called by various names such as proconvertin, stable conversion factor, co-thromboplastin, factor VII, SPCA, etc. Some believe that it may be regarded as a derivative or even a precursor of



thrombin. Like prothrombin, it is believed to require vitamin K for its physiological production, and Dicumarol decreases its concentration in plasma. It is not found in the most active preparations of purified prothrombin.

**Platelets.**—When platelets alone or platelets and calcium ions are added to solutions of purified prothrombin, thrombin is not produced. At least one other substance must be added. As already mentioned above, this may be from plasma or the fixed tissues. One of the substances of platelets functions in somewhat the same way as serum Ac-globulin and has, therefore, been referred to by the term platelet AcG. It seems likely that another substance is present in platelets, since certain patients have platelets that are not capable of participating in the activation of purified prothrombin in the same way as normal platelets.

**Neutralization of Thrombin.**—The antithrombin of plasma is also found in abundance in serum and is presumably a protein whose activity can be removed by ether extraction. It can destroy all the thrombin activity derived from prothrombin, and even thereafter much antithrombin remains in serum. This is remarkable because there is potentially 150 times more thrombin available from the prothrombin in 1 ml. of plasma than is needed to clot an equal volume of blood in 15 seconds. It has been suggested that antithrombin is associated with lipoprotein fractions of plasma, but thus far antithrombin has not been obtained in pure form. In addition to the antithrombin activity of plasma, a small amount of thrombin disappears by adsorption on fibrin. Furthermore, during the activation of prothrombin, another substance is produced which is concerned with the neutralization of thrombin activity. This is evidently different from antithrombin, which is present before coagulation begins. This latter observation is of recent origin and has not been studied extensively as yet.

The natural antithrombin activity of plasma is destroyed by ether extraction. After that there still remains the antithrombin-like activity attributable to heparin; i.e., heparin and a plasma co-factor together inhibit the action of thrombin. Thrombin, as a molecule, is apparently not altered by this mechanism, which is probably an interference phenomenon. Heparin alone is unable to function in this way and the exact nature of the co-factor of plasma is not known other than that it is a large molecule and presumably a protein. We may then account for the suppression of thrombin activity on the basis of four mechanisms.

- (1) Some thrombin is adsorbed on fibrin.
- (2) A factor in plasma neutralizes thrombin activity.
- (3) Heparin and a plasma co-factor interfere with the interaction of thrombin and fibrinogen.
- (4) During the activation of prothrombin, the antithrombin activity of plasma is greatly augmented.

**Sequence of Events.**—No one has as yet been able to set down the sequence of events in the activation of prothrombin, and with our present knowledge

that is not possible. Nevertheless, a valuable concept can be conveyed by presenting some of the most likely possibilities as follows:

- (1) Prothrombin  $\xrightarrow[\text{Platelet AcG}]{\text{Ca}^{++}, \text{Thromboplastin}}$  Thrombin
- (2) Plasma Ac-globulin  $\xrightarrow{\text{Thrombin}}$  Serum Ac-globulin
- (3) Prothrombin  $\xrightarrow[\text{Serum AcG}]{\text{Ca}^{++}, \text{Thromboplastin}, \text{Platelet AcG}}$  Thrombin
- (4) Prothrombin  $\xrightarrow{\text{Thrombin}}$  Thrombin
- (5) Prothrombin  $\xrightarrow[\text{Other activators}]{\text{Ca}^{++}, \text{Platelets}, \text{Platelet Co-factors}}$  Thrombin
- (6) Fibrinogen  $\xrightarrow{\text{Thrombin}}$  Fibrin + Fibrinopeptide
- (7) Fibrin  $\xrightarrow[\text{Fibrin stabilizing factor}]{\text{Ca}^{++}}$  Fibrin clot
- (8) Serum Ac-globulin  $\xrightarrow{\text{Thrombin}}$  Inactive Ac-globulin
- (9) Thrombin + Antithrombin  $\longrightarrow$  Inactive thrombin

These events are so complex that it is plain to be seen that this is capable of enormous variation from the quantitative point of view. The first equation is probably a slow reaction. Later there is great acceleration, as, for example, when active Ac-globulin is presumed to have formed. All the equations represent to a greater, and then to a lesser, extent active events occurring at the same time. The clot one sees is more or less incidental to the wonderful interplay which may last for an hour or so. Note that inhibitors are disregarded in the above presentation.

### **Inhibitors of Prothrombin Activation.—**

**HEPARIN.**—The concept of inhibitors of prothrombin activation was introduced by Howell, and heparin is perhaps the best known of this group of substances. This anticoagulant is a mucicetin sulfuric acid. The amino sugar is glucosamine, and the uronic acid is glucuronic acid. If it is present in normal blood, it must be in small amounts. On the other hand, it is abundantly present in the mast cells, from which it is released under special circumstances, such as anaphalactic shock and "peptone shock."

It acts as a powerful anticoagulant in conjunction with a co-factor presumed to be a plasma protein. This co-factor is required, for heparin alone does not inhibit the activation of purified prothrombin. It may be recalled from the preceding remarks that heparin also requires a co-factor to act as an antithrombin. Whether the two co-factors are the same is not known.

**ANTITHROMBOPLASTIN.**—This substance was first studied by Tocantins who has connected it with the problem of hemophilia. It is apparently a lipid that is quite generally distributed in tissues as well as in plasma. Its exact mechanism of action is not known.

Consideration may now be given the reasons for the action of the various coagulation preventives. Whipping, or defibrinating, blood really causes coagulation around a foreign object and therefore is not a method of preventing coagulation. Oxalates, citrates, and fluorides, of course, take calcium ions out of solution. The action of heparin has been discussed above. Bile salts are inhibitors of thromboplastin. Both heparin and hirudin prevent blood coagulation *in vivo* as well as *in vitro*. Polypeptides ("peptones"), however, act as anticoagulants only after injection into the living animal. They apparently stimulate the production of heparin by the body. The use of paraffined cannulas and receiving vessels and the application of cold seem to owe their virtues as preventives of coagulation to the fact that they tend to slow down the activation of prothrombin to thrombin by thromboplastin. The reason why the blood in a section of a carefully doubly ligated blood vessel does not coagulate is that no thromboplastin has been released. If some damage to the integrity of the vessel wall is done by the ligature or by the action of bacteria, thromboplastin will be produced and will enter the segment from the walls and the surrounding matrix of the injured vessel, and coagulation will ensue. Platelets clump or clot around the injury and then disintegrate and serve as coagulation centers. This agglutination of platelets does not depend upon fibrin formation and is mainly brought about by globulins. (Copley.) These platelet agglutinant factors are present in tissue juice, and thus the agglutination of platelets at the site of vascular injury can be explained.

**Clotting Time, Bleeding Time, and Prothrombin Time.**—Clotting time, bleeding time, and prothrombin time are determined in clinical laboratories to aid in diagnosis or to ascertain the state of the blood prior to surgical operations. Several methods are available for each. For *clotting time*, one method employs fine capillary glass tubes. These are filled from a large drop of blood which has exuded from a deep cut in the skin. At short intervals, pieces are broken off and the moment of coagulation is evidenced by the appearance of a thread of fibrin between the fragments as they are slowly separated. This test, although still popular, cannot be recommended because the admixture of tissue juice may accelerate considerably the coagulation time of whole blood. A *new test for blood coagulability* is based on the phenomenon that the coagulation time of whole blood may be delayed by dilution with physiologic saline. (Copley and Houlihan.) The blood sample is drawn into a syringe after about 1 ml. of blood have been taken into another syringe and discarded, in order to exclude admixture of tissue juice. The blood is discharged into a beaker. Now 1 ml. is pipetted into an empty tube, and another milliliter into a tube of the same size containing 1 ml. of physiological saline. After mixing this 10 per cent blood in saline sample, serial dilution is carried out twice more, care being taken to avoid bubble formation. These blood saline samples are incubated at 37°C. and the time of gelation is noted. Hypercoagulability is estab-



lished if all blood concentrations (100, 50, 25, 12.5 per cent) have short coagulation times, which approximate that of the 100 per cent concentration. Hypocoagulability can be manifested even though the 100 per cent blood concentration is within normal limits. In that case the 50 or 25 per cent blood concentration would exhibit markedly prolonged coagulation times. If *bleeding time* is desired, the blood exuding from the cut is removed at ten- to fifteen-second intervals by touching the cut with filter paper. When no spot of blood is seen on the paper, bleeding has stopped; the time is noted, and this is called the bleeding time. A better controlled bleeding time test was developed by Copley and Lalich. After infliction of a standard-sized wound, in the end phalanx of a finger, the emerging blood is allowed to flow freely into physiological saline kept at a constant temperature of  $37.5^{\circ}\text{C}$ . The bleeding time is noted with a stop watch from the instant of infliction of the wound until bleeding has stopped. *Prothrombin time* is an indirect and inverse measure of the amount of prothrombin present in blood; i.e., an increased prothrombin time means a lower level of prothrombin. In the method of Quick, blood is oxalated and centrifuged under standard conditions. To the oxalated plasma is added an excess of thromboplastin (usually an emulsion of rabbit's brain) and then  $\text{CaCl}_2$ . The time required for clotting to occur after the addition of the  $\text{CaCl}_2$  is taken as the prothrombin time.

Coagulation time of whole human blood is normally from two to ten minutes at  $37^{\circ}\text{C}$ ., depending upon the volume and size of the blood vessel. Increase in coagulation time, or bleeding time, may be due to diminution in the amount of any one of the diversified clotting factors, but bleeding time involves not only these, but also the amount of platelet agglutinant substance present in the cut tissues. The bleeding time is terminated primarily by a platelet agglutination thrombus. This clot may be mixed with a coagulation thrombus, in which fibrin may have precipitated or in which the blood has gelled. This clot, which was designated as wound thrombus, is actually a clot which seals numerous capillary vessel wounds. Moreover, in bleeding time determinations, the character of the clot plays a role, since a poorly adherent clot will be washed away by the flow of blood as rapidly as it is formed. In determining coagulation time the character of the clot is not noted—merely the time required to form any clot. By the prothrombin time technique, since an excess of thromboplastin and calcium ions are added and fibrinogen is always present, about the only variable is prothrombin.

There is little relationship among these determinations. The one condition in which coagulation time is prolonged is hemophilia. Therefore a normal coagulation time is not of much significance, while an increased one is. In hemophilia, the bleeding time is usually not prolonged. In purpuras, on the other hand, bleeding time is usually lengthened. Prothrombin time is generally normal in hemophilia, in the purpuras, and in many types of jaundice, but in obstructive jaundice and in conditions of marked involvement of the liver there is a low prothrombin level. Hemorrhage in the newborn infant and conditions leading to a diminished absorption of vitamin K also have lengthened

prothrombin time. When the *concentration of prothrombin* falls below 30 per cent of normal, the prothrombin time rises above twenty seconds, but *clotting time* is likely to remain normal until the prothrombin falls below 20 per cent of normal. This is why a patient with obstructive jaundice may have normal clotting time before an operation and suddenly have uncontrollable hemorrhages after the operation.

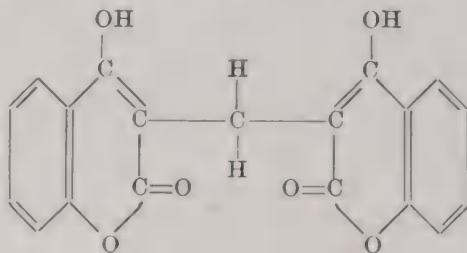
**Retraction Time.**—After normal blood clots, the clot contracts or “retracts” and separates from the fluid serum. This retraction is also termed *syneresis*. The time required for this to occur can be measured and is called “clot retraction time.” Normally this begins within a few minutes to an hour or two and is completed in from eighteen to twenty-four hours. For this determination 2 or 3 ml. of blood are drawn into a test tube and are incubated at 37° C. It is observed at hourly intervals and the time noted when complete retraction has occurred. In certain types of purpura the clot remains bulky and does not retract even after several days. In hemophilia, the clot, if it does form, has normal retractility. The rate of retraction may depend upon the number of platelets present but this is still a matter of controversy.

**Thrombi and Emboli.**—Despite the intricate mechanism which nature has devised to prevent blood from clotting until it is shed, this sometimes fails and clotting does occur within the blood vessels. Such intravascular clots, called thrombi, may arise if the blood vessel is damaged or if platelets or red blood cells agglutinate. A local excess of thromboplastin may thus arise from the tissue fluid of the vessel wall. Platelets are agglutinated by tissue juice. Each platelet agglutination thrombi may serve as coagulation centers and initiate fibrin formation and blood gelation. A diminution in the rate of blood flow, under other conditions, may contribute to thrombus formation. A thrombus may completely block a vessel at first, but as syneresis occurs, the retraction may permit the flow of blood past the clot. If the clot is not detached, it may eventually be organized or absorbed, with no harm resulting. However, if it is detached and is swept to some other location, it becomes an embolus. Emboli are often dangerous and even fatal, depending upon the site of the obstruction, the vessels of the heart and brain being particularly important from this standpoint. The administration of heparin or Dicumarol clinically to prevent thrombosis is a recent development. It appears to lower the incidence of thrombosis after operative procedures. In certain types of vascular surgery heparin is extremely useful.

Oral administration of heparin is ineffective. It must be given parenterally and, because of the transient effect of single doses, it may be given either by means of a continuous intravenous drip or intramuscularly. This results in the maintenance of a lengthened coagulation time. Heparin has been crystallized and no toxic effects have been experienced with the purified crystalline material. The great danger is the possibility of hemorrhage during an extreme prolongation of the coagulation time. Since heparin is expensive and must be given parenterally, Dicumarol, which is inexpensive and can be given orally, represents a great advance. When given by mouth, when the sodium salt is injected intravenously, it prolongs the prothrombin time. Its effect is slow, requiring from one to three days after the start of

treatment, and frequent determinations of prothrombin time must be made in order to control the dosage correctly. Some individuals are more resistant and some more susceptible to this drug.

The "hemorrhagic disease" of cattle was found to be due to the ingestion of spoiled sweet clover hay in 1922. In 1940-1941 Link and his associates isolated the causative agent, Dicumarol, identified it, and synthesized it. Its formula is:



Dicumarol

3,3' methylenebis (4-hydroxycoumarin)

It is a colorless crystalline solid, almost insoluble in water, acids, and in most organic solvents. It forms soluble salts with strong alkalis. In spite of its insolubility in water, it is readily absorbed from the gastrointestinal tract. It has no effect on blood clotting *in vitro*, and the mechanism of its action is unknown. However, its action is antagonized by vitamin K and therefore it is suggested that it inhibits vitamin K and thus either depresses the synthesis of prothrombin or an altered prothrombin is synthesized.

**Lysis of Blood Clots.**—Although clots are quite stable, they can be removed or dissolved very slowly. The mechanism involved bears some resemblance to the clotting mechanism. A substance in plasma called "pro-fibrinolysin" is activated by "fibrinokinase," present in many tissues. The resulting agent is an enzyme, "fibrinolysin," or "plasmin," which can dissolve fibrin. Fibrinokinases have also been obtained from certain bacteria. According to Astrup and co-workers, two proenzymes seem to be present in human plasma, one of which is activated by fibrinokinase and the other by streptokinase. Fibrinolysin can be inactivated by normal plasma. The anti-fibrinolysin, which causes this inactivation, is increased in amount in various pathological conditions.

## ANEMIAS

Those conditions in which the number of red cells or the amount of hemoglobin is reduced below normal are termed "anemias." There are a number of types of anemias which can only be briefly considered here. Anemias are due to (1) loss of blood, (2) destruction of blood, or (3) defective formation of blood. The first group includes acute and chronic hemorrhage. Destruction of red cells is brought about by hemolytic poisons, which may be of bacterial or metabolic origin, a result of jaundice, or due to the absorption of industrial poisons. Anemias of the third group include hypochromic anemias, pernicious anemia, and aplastic anemias.

A hypochromic anemia, i.e., one in which red blood cells contain less hemoglobin than normal, may be experimentally induced in animals by feeding them exclusively on milk. The lack of iron and copper in this food is un-



oubtedly the cause of this condition, for otherwise milk is a superior food. Similarly in man an iron-deficient diet may give rise to an anemia ("hypochromic" anemia), in which the hemoglobin content of the blood is reduced to a greater extent than the number of red cells. The cells not only contain less hemoglobin, but may also be reduced in size. A similar anemia of infants is not uncommon. As stated before, the infant comes into the world with a rich store of iron. However, this store is accumulated toward the latter part of gestation. Hence a prematurely born baby may not have enough iron to take him over the period during which the diet is exclusively milk, and anemia may result. In young women anemias may occur due to a combined effect of malnutrition and menstrual bleeding. This type, called *chlorosis*, is not as common now as it formerly was. Other conditions in which hypochromic anemias sometimes develop are pregnancy and various infectious diseases. Hypochromic anemias are treated mainly by the administration of inorganic iron.

Pernicious anemia is due to an inability to form red blood cells, not to any difficulty in synthesizing hemoglobin. There results a great diminution in the number of red cells and consequently in the percentage of hemoglobin. The color index, however, is high and the blood picture is quite abnormal. (See page 590.) The red bone marrow is greatly increased in volume, displacing the yellow marrow and sometimes even invading the true osseous tissue. This greater amount of unused hemoglobin causes a rise in iron and bilirubin in blood plasma, the latter apparently in the colloidal state, since an indirect van den Bergh reaction is observable. There is invariably a lack of HCl in the gastric juice, a fact of great importance in aiding in diagnosis and of interest in explaining the mechanism of the condition.

As might be expected, administration of iron salts to patients with this disease is of no avail. The treatment is based on the results of brilliant experimental work of a number of investigators. The first step was the work of Whipple and his co-workers. They produced a severe anemia in dogs by repeated bleedings and studied the influence of diet upon blood regeneration. It was discovered that beef liver was the most effective food in this respect. This led Minot and Murphy to administer liver in large amounts to patients with pernicious anemia and they noted remarkable improvement. Liver may not only be fed, but preparations are also available for parenteral injection.

In following the effect of treatment, the physician observes the proportion of reticulated red cells in the blood. These "reticulocytes" are formed by over-stimulated marrow and are so-called because their protoplasm shows a delicate network or reticulum which stains with basic dyes. They represent immature stages of development of the erythrocytes, and their numbers furnish an index of the rapidity of blood regeneration. This index enabled Castle and colleagues to estimate the curative influence of a number of preparations, and this work threw light upon the mechanism of the action of liver. These experiments were performed on patients with pernicious anemia. Raw lean beef fed to patients with pernicious anemia had no effect on the anemia. However, when raw beef digested with normal human gastric juice was administered to the patient through a stomach tube, the effect was comparable to

feeding liver. Gastric juice alone had no beneficial effect. These and other experiments seemed to indicate that normal gastric juice contains a factor, termed the "intrinsic" factor, which reacts with the "extrinsic" factor, found in foods, to produce the antianemia or hemopoietic principle. This may be linked to the fact that another symptom of pernicious anemia is a lack of both HCl and pepsin in the gastric juice due to atrophy or degeneration of the fundic portion of the gastric mucosa. It is known that this portion of the stomach which atrophies in pernicious anemia normally produces the intrinsic factor. The intrinsic factor is thermolabile and is considered by Glass to be the "glandular mucoprotein" of the gastric juice, or a substance closely related to it. The extrinsic factor is found in various foods, notably beef muscle, beef heart, rice polishings, and wheat germ. It is thermostable and is considered to be identical with vitamin B<sub>12</sub>.

Vitamin B<sub>12</sub> is far more effective when given parenterally than by mouth, unless normal human gastric juice is also administered orally. Therefore, it is believed that the intrinsic factor either facilitates the absorption of vitamin B<sub>12</sub>, or in some way increases the activity of the vitamin or protects it from destruction. Furthermore, it is possible that there may be no "antianemic factor" formed in the liver by the interaction of the extrinsic and intrinsic factors but that vitamin B<sub>12</sub> may be the effective antianemic principle through its effect in converting folic acid to folinic acid, and the intrinsic factor (in gastric juice) is necessary for its absorption. (See also pages 300-304.) It must be remembered that in the production of red blood cells, there is the further need of the required amino acids, iron, and copper. The vitamin is highly effective for relieving not only the hematological symptoms, but also the lingual and neurological symptoms. This is not true of "folic acid," the vitamin which was used before B<sub>12</sub> was discovered, and which benefits only the anemic phase. Pteroylglutamic acid (folic acid), however, is very efficacious in the treatment of the anemic phase of pernicious anemia and of the macrocytic anemias of pellagra, of pregnancy, and of sprue. They are nutritional in origin, in part at least, and thus constitute a group of anemias not amenable to treatment with iron.

Sickle-cell anemia is an interesting condition which appears to be of a hereditary nature. It occurs only in the Negro race. About 8 per cent of American Negroes have the "sickle-cell trait," but only about 1 in 40 of these develop the severe chronic anemia. In this disease the erythrocytes undergo reversible changes in shape to crescent and other forms in response to variations in the partial pressure of oxygen. Pauling has shown that this is due to an abnormality of the hemoglobin itself, located in the globin portion of the molecule. Those individuals who have the trait, but not the anemia, possess some of this abnormal and some of the normal hemoglobin.

## BLOOD TRANSFUSION AND BLOOD SUBSTITUTION

Loss of blood as a result of hemorrhage or shock is treated by blood transfusion, or by the injection of a substitute for blood. Great activity was shown in this field even before World War II, and it has redoubled since. Blood



transfusion, or infusion of a substitute, sometimes preceded by the removal of blood, has been used in a number of conditions other than hemorrhage and shock. In general, the purpose is to restore blood volume, to increase the colloidal osmotic pressure, or to provide nutritive or immunological factors.

Whole blood, either citrated or heparinized, is, of course, the material most approved, being most physiological. Care must be taken that the blood used is suitable. Not only must the donor be a healthy human being, but the blood must be compatible with that of the recipient.

*Blood Groups.*—It is well known, even to laymen, that all human blood is not alike, but that four different major types or groups exist, and that transfusion of blood of one type into an individual whose blood is of a different type may have dire results. We owe much of our knowledge of blood grouping to Landsteiner and his co-workers. In human red blood cells there occur two possible major antigens, which are proteins. In the serum there may be two possible corresponding antibodies, which are also proteins—modified serum globulins. If cells containing one of these antigens are suspended in serum containing the corresponding antibody, the antibody unites with the antigen, and as a result the red cells agglutinate at first and then may undergo lysis. If such a reaction were to take place between the cells and serum of an individual's own blood flowing through his blood vessels, it would be incompatible with life. Therefore, it is obvious that no one can have in his serum antibodies which correspond to the antigens in his own red cells. If antigen and antibody are not specific for each other (i.e., do not correspond), they will never combine and, therefore, can exist in the same individual without any ill effect. Of the two major antigens and antibodies which occur in human blood, there are four possible combinations which can exist without reactions, and all four do exist in man. They represent the four major blood groups, which are usually designated as Groups A, B, AB, and O. In addition to these major groups, certain subgroups are known to occur. The composition of the blood of these major groups is as follows:

BLOOD GROUP	ANTIGENS IN CELL	ANTIBODIES IN SERUM
A	A	b
B	B	a
AB	A and B	—
O	—	a and b

Serum of Group A will agglutinate corpuscles of Groups B and AB; Group B serum will agglutinate Group A and Group AB corpuscles; Group AB serum will not agglutinate corpuscles of any group; and Group O serum will agglutinate corpuscles of all other groups.

Because of these differences in antigen and antibody content of the blood, and the reactions which occur between them, an individual can safely receive by transfusion only those types of blood which will not react with his own. Before a transfusion is given, a cross-matching of the bloods must always be done; i.e., a testing for possible agglutination between patient's cells or serum and donor's serum or cells. If any agglutination takes place, the bloods are "incompatible."

To determine the blood group of a person, samples of his red cells are mixed with samples of known Group A serum and known Group B serum, and the mixtures are observed for agglutination. By the reactions which occur, any blood group can be identified. Blood types are hereditary, and therefore can sometimes be used as a basis for determining the paternity of an individual. Also of value in this connection are certain other antigens which have recently been recognized in red blood corpuscles, the M and N antigens. All people have, in addition to the antigens mentioned above, either the M or N antigens or both (MN). For these antigens there are no corresponding antibodies which occur naturally in the serum. Such antibodies can, however, be induced to form in rabbits by injecting them with human red cells known to contain either the M or N antigen. The antibody will appear in the rabbit's serum. If the serum is removed from the animal and mixed with human cells containing the corresponding antigen, it will agglutinate those cells. Thus this serum, contain-



ing known M or known N antibodies, can be used to detect the presence of M or N antigens in human cells. A third group of antigens known as the *Rh* factors is also present in the cells of about 89 per cent of human beings. For these factors, again, there are no naturally occurring antibodies in the serum, but they can be produced in animals, or in man, under certain circumstances. All of these various groups of antigens are hereditary, and the several groups are transmitted independently of one another. Consequently there are actually several hundred possible combinations of them in human blood cells. The presence of any of the antigens can be detected by mixing the cells with serum containing known antibodies. In attempting to determine the possible paternity of a child, use is made of all of these antigens.

The Rh factor is important in another respect. Although there are no naturally occurring antibodies for this antigen, if Rh-positive cells are transfused into an Rh-negative individual (one whose cells contain no Rh antigen), antibodies may form in the recipient as a *result* of the transfusion. Then later transfusions of Rh-positive blood into this same person may give rise to reactions. Similarly, an Rh-positive fetus developing in an Rh-negative mother may also result in the formation of Rh antibodies in the mother. In subsequent pregnancies these Rh antibodies of the mother, by passing into the blood of an Rh-positive fetus, may give rise to severe blood reactions in the child, a condition known as *erythroblastosis fetalis*.

Substitutes for whole blood include saline solutions, foreign colloids, and plasma or fractions of plasma. The injection of isotonic sodium chloride, or mixtures of salts such as Ringer's or Locke's solution, is of little value in restoring blood volume, since the salts diffuse freely and consequently the fluid is quickly lost. However, in cases of dehydration, in which the blood becomes concentrated through loss of water,\* such solutions may be given and are more likely to be retained. Of the foreign colloids tested, the best is said to be dextran, with gelatin next, then acacia, and last Periston (polyvinylpyrrolidone). (Knutson.) None of them is entirely satisfactory as a blood substitute and sometimes severe reactions follow their use. As regards plasma or fractions of plasma, one would expect that serum would be the best blood substitute since no anticoagulant need be added to it. This is not the case, however. Blood serum frequently produces marked reactions and is not generally used for this purpose. Serum albumin has lately been tested and promises to be a very useful agent. It makes up about 62 per cent of the blood proteins and exerts about 85 per cent of the colloidal osmotic pressure of the plasma. Because of its great solubility it may be given in concentrated form. A unit volume of about 100 ml. containing about 25 grams of albumin is being used and is the osmotic equivalent of 500 ml. of plasma.

Blood plasma has proved to be the most practical blood substitute and, in fact, is more effective than whole blood in most conditions of loss of blood *fluid*—not, of course, if there is loss of whole blood. Blood of normal human beings is collected, citrated, and pooled, all under rigidly aseptic precautions. It is then centrifuged at from 2 to 4° C. and the plasma from a large number of bleedings is pooled. (The red blood cells, it has recently been found, may be used as material for surgical dressing of wounds.) The plasma may be preserved in the liquid condition, if properly refrigerated, or it may be frozen

\*The degree of concentration of blood may be determined by means of the *hematocrit*. This is simply a graduated tube of small bore, which is filled with oxalated blood to a definite mark and centrifuged under standardized conditions. The volumes of formed elements and plasma may then be read off directly. The relative size of the average corpuscle present may also be computed if a blood count is made on the same sample.

r dried. The modern method of drying is the most practical and most widely used. It consists of rapidly freezing the plasma in rotating bottles. This fixes the solid plasma as a shell. It is then dried, while frozen, under greatly reduced pressure. This is called the lyophile process. Other biological products have been preserved similarly. Such desiccated plasma may keep for years. The proteins are not denatured to any great extent and the immunological properties are essentially unchanged. The mixing of the plasma from many individuals obviates the necessity of considering types of donors and recipients. The reasons for this are (1) that pooling causes a dilution of the various specific agglutinins, and therefore the agglutinating power is much weaker, and (2) that no red cells are present in the plasma, which might be agglutinated, if the plasma of the recipient is incompatible. The lyophilized dry flaky plasma is kept in sterile containers under vacuum until needed. All that is necessary for use is the addition of the required amount of sterile distilled water. The containers are so arranged that they can be easily manipulated.

Great progress has been made by Cohn and associates in fractionating the proteins of plasma so that they may be studied and put to clinical use. The methods of separation are based on complex physico-chemical principles. For example, some proteins form dissociable complexes with each other, with smaller bipolar ions, with complex organic molecules, or with certain heavy metal and alkali earth ions. These characteristics aid in fractionation procedures. Furthermore, small amounts of ethanol lead to considerable differences in solubility of proteins. By utilizing these properties, there have been separated from human plasma a series of protein products, each a stable white powder responsible for a different natural function. The fractions, with their uses, include:

1. Albumin, which is being used instead of dried whole plasma for the reasons mentioned.

2. Immune globulin, or gamma globulin, which has proved of value in the prevention and treatment of measles. Recently it has been found to give temporary safeguard against poliomyelitis, or to lessen the crippling effects of that disease.

3. Agglutinins, for blood typing.

4. Fibrinogen, obtained in pure form, which can be made into plastics that have application in surgery.

5. Thrombin, which, together with fibrinogen, of course, yields fibrin. Fibrin films have been prepared which can substitute for natural membranes, and fibrin foams can be used, with thrombin, to accelerate blood clotting in operative work.

The presence and relative proportions of the individual proteins have been demonstrated by the electrophoretic method. Thus, in human plasma there has been found 62 per cent albumin, which is the fastest moving component, 7 per cent  $\alpha$ -globulin, 13 per cent  $\beta$ -globulin, 12 per cent  $\gamma$ -globulin, and 6 per cent fibrinogen. The  $\gamma$ -globulin moves most slowly in the electric field. The fibrinogen molecules are long, rodlike in shape, and when flowing in the blood vessels



are assumed to orient themselves parallel to one another, as do logs in a rapidly flowing river. When clotting occurs the denatured molecules may be imagined as being unbalanced in some way, so that they no longer can keep in position and a "log jam" occurs. The molecular weight of fibrinogen of human blood is about 500,000. It is insoluble in distilled water but soluble in dilute salt solution, thus resembling globulins in solubility. The  $\gamma$ -globulins are of great importance since they possess the immune properties of blood. The  $\beta$ -globulins are highly colored and seem to have prothrombin associated with them.

The infusion of plasma or plasma proteins has been recommended for many conditions, some of which may be mentioned briefly.

It is usually agreed that in shock there is loss of plasma through the capillary walls into the tissue spaces. This leads to a decrease in blood volume, hemoconcentration (i.e., concentration of formed elements), and lowered colloidal osmotic pressure. In severe and extensive burns there is a great loss of proteins from the blood because of transudation of fluid at the site of the burn. At the same time there is believed to be an absorption of toxic substances formed at the burned tissue. These toxins cause an increase in capillary permeability throughout the body and more plasma is lost. After extensive burns the loss of blood plasma may be even greater than in shock. When hypoproteinemia occurs, as it does in a number of clinical syndromes, plasma may be administered. Loss of blood as a result of hemorrhage, while best replaced by whole blood, may also be replaced by plasma, and this is perhaps its most general use.

The treatment of shock by the intravenous injection of whole blood, plasma, blood proteins, or other colloids is not accepted by all investigators as the correct method. Foremost among this group is Allen, who insists that large volumes of physiological saline will give as good results.

All of these procedures are, in a sense, emergency measures. It should be borne in mind that the best way, the most physiological way, to replace blood is by enabling the organism to replenish it in the normal manner. A regimen to accomplish this should follow all transfusion methods. Blood proteins contain large amounts of histidine, lysine, and threonine. Hence proteins, rich in these three essential amino acids, should have a prominent place in the diet. It may also be advisable to administer a mixture of the amino acids themselves orally or parenterally to hasten the formation of the natural blood proteins by natural processes. Other dietary measures should be taken, including the administration of iron, copper, and vitamin supplements.

## LYMPH

Since the lymphatic capillaries drain the tissue spaces, the fluid present in both is similar. These fluids resemble blood plasma in composition, the chief difference being that blood plasma contains a higher percentage of protein than does lymph and tissue juice. This has been mentioned before as the reason for the colloidal osmotic pressure of the blood plasma being higher than that of the tissue fluids, while the crystalloidal osmotic pressure is about the same. The albumin : globulin ratio is higher in lymph than in plasma.



This is so because albumin, with a smaller molecule, diffuses from plasma into lymph more readily than globulin does, although neither diffuses freely. A smaller amount of fibrinogen is present, some prothrombin, and many leucocytes. It clots very slowly. The lymph of the thoracic duct has a higher concentration of protein than that of the lymphatic capillaries but lower than that of plasma; in other respects, during the fasting state, it also tends to resemble plasma. Since it drains the abdominal viscera, however, its composition changes with the state of digestion. After a meal, the fat content rises, since more than half of the fat absorbed goes by this route. In fact the lymph, or chyle, is decidedly milky if the food contains much fat.

## OTHER BODY FLUIDS

Considerable progress has been made in determining whether the various body fluids are dialysates (ultrafiltrates) of blood plasma or true secretory products. In accordance with the Donnan equilibrium, an ultrafiltrate will have a different distribution of the electrolytes from plasma, whereas the nonelectrolytes, such as glucose and urea, will be in the same concentrations. This is true of lymph, pleural fluid, peritoneal fluid, synovial fluid, and pericardial fluid. In cerebrospinal fluid and in the aqueous humor of the eye, analyses indicate that simple dialysis is supplemented by some selective secretion. For instance, glucose is lower in cerebrospinal fluid than in the blood, even in hyperglycemia, and the other nonelectrolytes vary in their concentrations from those of the blood plasma. The protein content of all these fluids is lower than that of blood plasma, and the ratios of the various proteins differ.

## CEREBROSPINAL FLUID

Normal cerebrospinal fluid is a clear, colorless fluid, having a specific gravity of from 1.004 to 1.008. It has an extremely low protein content with no fibrinogen and, as already stated, differs considerably from blood plasma in its concentration of nonelectrolytes. Its pH, however, is about the same as that of blood; namely, pH 7.35 to 7.40. Pathologically the fluid may be

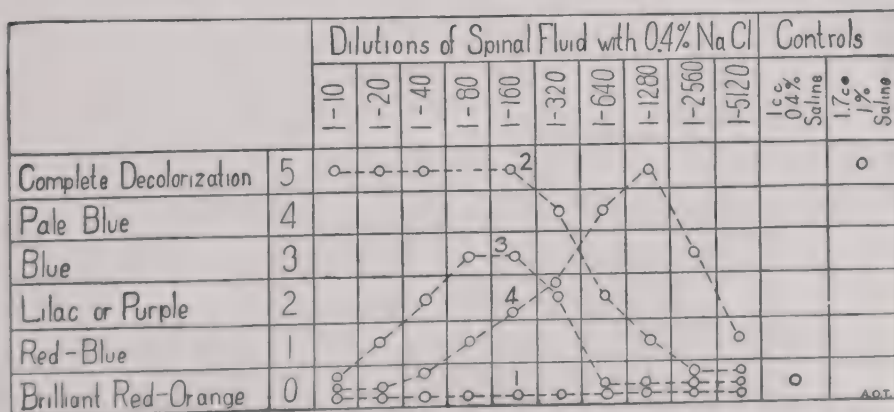


Fig. 24.—Types of reactions in the colloidal gold test. 1, Normal cerebrospinal fluid (no reaction); 2, parietic type; 3, syphilitic or tabetic type; 4, meningitic type. (From Todd, J. C., and Sanford, A. H.: *Clinical Diagnosis by Laboratory Methods*, ed. 10, Philadelphia, 1944, W. B. Saunders Co.)

increased in amount and, as a consequence, be under great pressure. In many of these conditions the protein content increases appreciably. It is usually referred to in clinical tests as the globulin fraction, since this seems to be the chief constituent to show an increase. Besides various quantitative and qualitative procedures of the usual type, Lange's colloidal gold test is also employed. The exact nature of the substances responsible for this reaction is unknown, but they are probably of a protein nature. The procedure consists in mixing cerebrospinal fluid in progressively increasing dilutions with a colloidal gold solution. Normal fluid causes no change in the appearance of this orange-red colored solution. Fluids from certain pathological conditions produce changes in this color, depending upon the particular condition and the dilution. When these results are plotted, they produce curves which are rather characteristic and thus aid in diagnosis. In Fig. 24 are shown some of the curves obtained by this test.

### SEMEN

The study of the composition of semen has assumed greater interest in recent years because of its possible bearing on the problem of infertility (Weisman). Most of the work has been done on the seminal plasma, the fluid in which the spermatozoa are suspended. The spermatozoa are constituted largely of nucleoproteins, which differ in various species as regards their isoelectric points, amino acid make-up, etc.

Human seminal plasma is a mixture of the secretions of a variety of glands and tubular epithelial linings. This may account for the great differences in analytical figures reported in the literature. The pH is about the same as that of blood plasma, as is the  $\text{CO}_2$  content. Chloride and cholesterol are much lower, whereas phosphorus and lactic acid are much higher. The high phosphate is undoubtedly of importance in buffering any acid present in the female secretions. Calcium, urea, and sugar are about twice as high in semen as in blood. It is interesting that the sugar present is fructose rather than glucose. (Mann.) The analyses of proteins are most discordant, both qualitatively and quantitatively, but the most recent work by electrophoretic methods indicates that the protein fractions are qualitatively identical with those of blood serum. (Gray and Huggins.) From these facts it would appear that seminal plasma is not an ultrafiltrate, and indeed its derivation from so many sources would lend support to that hypothesis.

### TRANSUDATES AND EXUDATES

The fluid formed by passage through a membrane is called a transudate. A fluid deposited in or on a tissue is known as an exudate. Actually the difference between a transudate and an exudate is difficult to define. If inflammation exists, the fluid is an exudate. Thus a transudate may be a normal fluid, such as lymph, or it may be a pathological fluid, such as some sterile ascitic fluid (e.g., peritoneal). From a physical and chemical standpoint, transudates have a low specific gravity (below 1.015), a low protein

content, and clot more slowly than do exudates, if at all. Exudates have a higher specific gravity (above 1.018) and a higher protein content (above 3 per cent) than have transudates and clot rapidly. However, in some rare instances in which these physical and chemical features tend to merge, it is difficult to determine whether a fluid is a transudate or an exudate.

## MEDICOLEGAL TESTS FOR BLOOD

It is frequently necessary to determine whether stains or smears are composed in whole, or in part, of blood. If the material is fresh and an isotonic suspension is made, it is sometimes possible to observe the red blood cells microscopically. The spectroscope can also be used, because the absorption spectrum of hemoglobin is quite specific. The various tests for the catalytic oxidizing effect of heme, such as the guaiac, benzidine, and reduced phenolphthalein tests, are very helpful, but are not specific for blood. Blood reacts after it is heated, as a result of the catalytic action of iron, as well as before, as a result of the enzyme peroxidase. Raw milk, pus, saliva, and other biological materials contain peroxidases which react similarly, but no reaction is seen after heating. Certain salts also give the guaiac test. If hemin crystals can be prepared, they are quite indicative of blood; this is Teichmann's test. (See Fig. 22.)

However, none of these tests are diagnostic of the species. An immunological reaction is necessary if this is to be determined. The test is based on the fact that the blood serum of an animal, into which has been injected repeatedly the blood serum of an animal of another species, gradually acquires the property of producing a precipitate when mixed with serum of an animal of the species whose serum was injected. This "precipitin reaction" is performed essentially as follows for the detection of human blood: increasing amounts of human blood serum are injected every four days into a rabbit until from 25 to 35 ml. have been administered. Four or five days later, the rabbit is bled and the serum obtained. This is preserved in sterile containers until a test is to be made. Such serum will form a precipitate if mixed with blood serum of human beings and of no other species.

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## Chapter 9

# ENZYMES

A catalyst is a substance which will accelerate a reaction but which is not itself altered by the reaction. An enzyme is a heat-labile organic catalyst produced by a living cell but which acts independently of the cell. Most vital reactions are enzyme reactions. Indeed, as Willstätter has well said, "Life is a system of cooperating enzyme reactions." Digestion, the building up and breaking down of tissues, cellular respiration, and muscle contraction are examples of physiological activities of paramount importance, all dependent on enzyme action.

It should be noted that not all biological catalysts are enzymes. Tauber places the biochemical catalysts in two categories:

1. Specific, cell-independent, biochemical catalysts or enzymes. These are destroyed by heat. Examples: pepsin, amylase, oxidase.
2. Specific, nonenzymic biochemical catalysts. These act mainly *in vivo*. They may or may not be destroyed by heat. Examples of these are genes, hormones, and viruses.

### History of Enzyme Chemistry

From prehistoric times man has observed four chemical changes which apparently occur spontaneously. They are the fermentation of sugar with the production of alcohol, the souring of milk, the souring of wine, and the production of ammonia in urine. Each is due to the growth of microorganisms which convert the substances present into other substances. All are now known to be brought about by enzymes, but for a long time it was felt that these reactions were bound up in the life cycle of the organisms and could only occur if these forms were present and living. In 1833 Payen and Persoz precipitated the starch-digesting enzyme from malt extract by means of alcohol. They named it "diastase" and compared it with the natural "ferments" which caused the souring of milk or of urine. It was at about this time that Beaumont recognized that the digestive action of gastric juice was due to a chemical substance, and in 1836 Schwann isolated this substance and named it "pepsin." Leuchs and Claude Bernard studied the digestive agents of saliva and pancreatic juice, respectively, in this period. The term "ferment" was used for a long time for any agent which would bring about a chemical reaction in biological material. Pasteur showed that many of these reactions were caused by the growth of microorganisms and he differentiated between "organized ferments" and "unorganized" or "soluble ferments"; i.e., between microorganisms and nonliving substances like pepsin. Later, Kühne introduced the term "enzyme" to mean a biological catalyst. However, it was not until 1897 that it was shown by Buchner that the microorganisms produce their effects through the agency of intracellular enzymes.



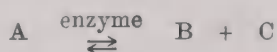
Recently, enzyme chemistry has progressed very rapidly. In 1926 the first enzyme was crystallized by Sumner (urease) and a number of others have been crystallized since then. This has enabled more exact studies of the action of the enzymes to be made—the kinetics, specific points of attack on the substrate, etc. The “coenzymes” were isolated in 1933 and later their structure was determined. Investigations centering on them has given a great deal of information regarding carbohydrate metabolism and cellular respiration.

Much has been written about the mechanism of enzyme action. However, it will suffice to say that the two main theories are as follows:

1. *Bayliss' hypothesis.* Since the enzyme is a colloid, it has an enormous surface. The substrate, or substance acted upon, is adsorbed upon this surface. When the reaction takes place at the interface. In this respect the enzymes are assumed to resemble inorganic catalysts like colloidal platinum, in which the reaction takes place at the surfaces of the many colloidal particles.

2. *Michaelis' hypothesis.* This is a chemical view as contrasted with Bayliss' physical approach. Michaelis believes that the catalyst reacts with the substrate, forming an intermediate compound. The latter then decomposes into the new products plus the original enzyme.

Like other catalysts, enzymes increase the rate of reaction but do not change the final equilibrium. That is, after the reaction



has reached equilibrium, the addition of more enzyme will not change the relative concentrations of A, B, or C. The rapidity with which this equilibrium has attained in the first place may be altered by adding to, or subtracting from, the amount of enzyme.

Although enzymes are catalysts, they differ from inorganic catalysts in some respects. The latter frequently catalyze many kinds of reactions. Enzymes never do, but they are rather specific in that they act only upon certain types of substances. Inorganic catalysts are unchanged by the reaction which they catalyze. They often may be recovered quantitatively and regenerated at the end of the reaction. Not so the enzymes, which are destroyed to a greater or less extent during their reactivity.

### Preparation of Enzyme Material

For the extraction of enzymes from biological material, usually a preliminary disruption of the cells is necessary. This is accomplished by grinding with sand, chopping, high pressure, autolysis (self-digestion), or desiccation followed by pulverization. Material so treated is extracted by one of a variety of solvents, depending upon the enzyme and the material. Glycerol, alcohol, buffers, saline, dilute acids, or alkalies all have been used in various percentages.

Often the enzyme may be studied in these extracts without further procedure. However, to concentrate and purify it any one of a number of methods may be employed. Willstätter and his pupils used the adsorption method.

This is based on the fact that the enzymes are colloids and may be adsorbed on other colloids such as kaolin or certain aluminum hydroxides. The enzyme is then released or "eluted" from the combination by mild chemical reagents. Others, especially Northrop, have used fractional salting-out methods to precipitate and concentrate the enzyme, sometimes getting rid of the salt by dialysis. The combination of treatment with butanol followed by differential salting out has recently been introduced and in many cases has proved highly successful (Morton). The preparation of highly purified enzymes is a long and tedious process, but the results of such experimentation have yielded extremely powerful enzyme preparations, a number of which have been crystallized. These include:

Urease (Sumner, 1926)	Catalase (Sumner and Dounce, 1937)
Pepsin (Northrop, 1930)	L-Glutamic acid dehydrogenase (Euler, et al., 1938).
Pancreatic amylase (Caldwell, Booher, and Sherman, 1931)	Carbonic anhydrase (Scott and Fisher, 1942)
Trypsin (Northrop and Kunitz, 1932)	Muscle phosphorylase (Green and Cori, 1943)
Yellow respiratory enzyme (Theorell, 1934)	Rennin (Hankinson, 1943)
Chymotrypsin (Northrop and Kunitz, 1935)	Hexokinase (Berger et al.; Kunitz and McDonald, 1946)
Chymotrypsinogen (Kunitz and Northrop, 1935)	Inorganic pyrophosphatase (Kunitz, 1952)
Carboxypolypeptidase (Anson, 1935)	
Pepsinogen (Herriott and Northrop, 1936)	

Some of these crystalline enzymes are shown in Fig. 25.

### Chemical Nature of Enzymes

The enzymes are protein in nature. This is generally accepted, although until quite recently it was thought that there might be some exceptions. Some enzymes consist wholly of protein, others contain some metallic ion, and still others are similar to conjugated proteins, the prosthetic group of which is heat stable and dialysable and is mainly responsible for the catalytic activity. The protein portion, however, is always required for enzymic action. It is often called "apoenzyme" and the prosthetic group, the "coenzyme," the two together forming a "holoenzyme."

Examples of the first type of enzyme, which is composed solely of protein, are pepsin, trypsin, and urease. Carbonic acid anhydrase contains zinc; tyrosinase, laccase, and ascorbic acid oxidase are copper-protein complexes; and catalase, peroxidase, and cytochrome oxidase are examples of iron-containing enzymes.

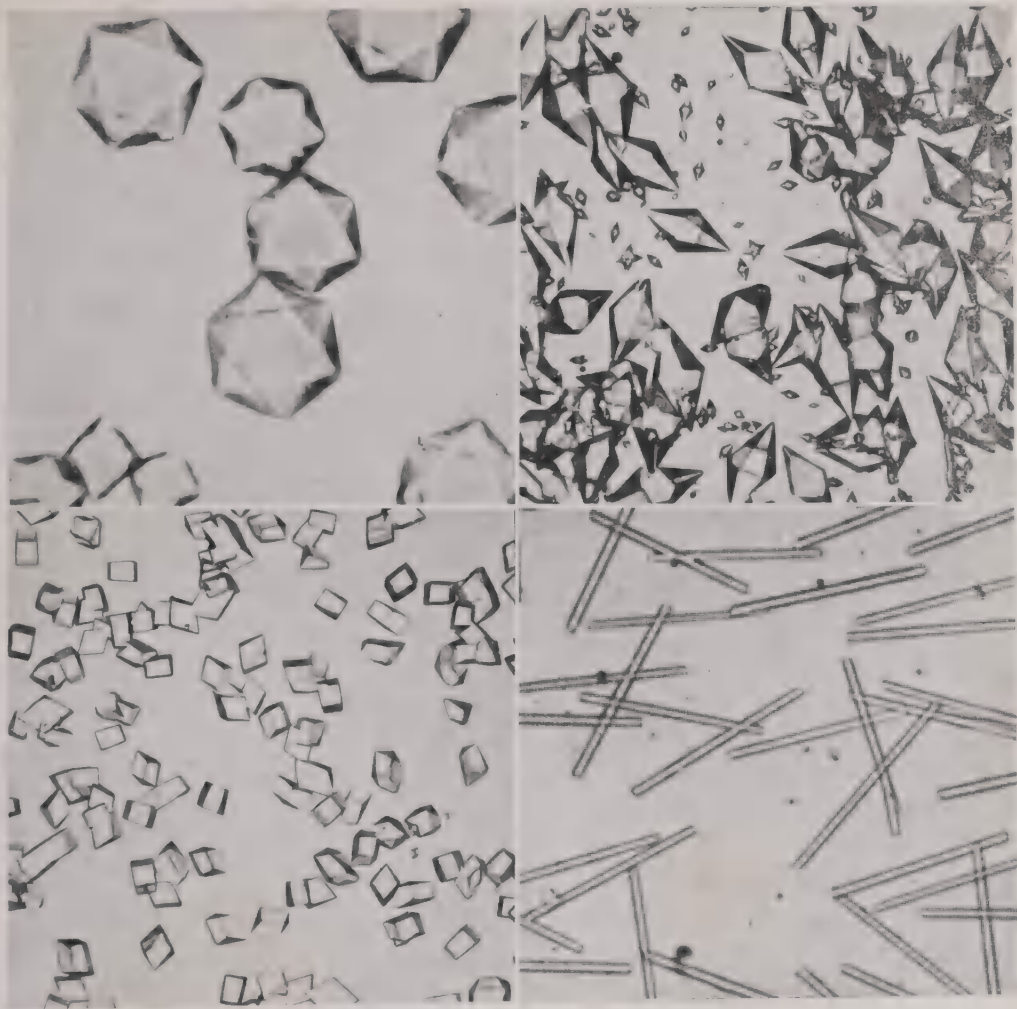
### Terminology of Enzymes

The enzymes which have been known for a long time are still known by the names originally given them; i.e., ptyalin, pepsin, trypsin, papain. In general, however, the suffix "ase" is affixed to the name of the substance acted upon. Thus, we have lipase, amylase, protease, and sucrase which act upon fat, starch, protein, and sucrose, respectively. Often the source is indicated, as, for example, pancreatic lipase. The adjective suffix "lytic" is also used in designating an enzyme; for example, the proteolytic enzyme of the gastric juice. The sub-

stance acted upon is called the "substrate." A "proenzyme" or "zymogen" is the inactive form of the enzyme present in the cell and sometimes in the secretion; e.g., pepsinogen and trypsinogen. It must be activated before becoming a true enzyme. This may be accomplished in some instances by a suitable hydrogen-ion concentration. In others, specific activating enzymes, or "kinases," are required. Furthermore "activators" or "coenzymes" are needed by most

A.

B.



C.

D.

Fig. 25.—Some crystalline enzymes. A, Crystalline urease (Sumner). (From Sumner, J. B., and Somers, G. F.: *Chemistry and Methods of Enzymes*, New York, 1943, Academic Press, Inc.)

B, Crystalline pepsin ( $\times 90$ ). (Courtesy Dr. John H. Northrop.)

C, Crystalline chymotrypsin ( $\times 123$ ). (Courtesy Dr. Moses Kunitz.)

D, Phosphorylase crystals prepared from rabbit muscle ( $\times 135$ ). (From Green, A. A., and Cori, G. T.: *J. Biol. Chem.* 151: 21, 1943.)

enzymes. There is not complete agreement in the use of these terms. However, the distinction usually drawn between an activator and a coenzyme is that the former is inorganic and the latter organic. An "antienzyme" is a biological substance which inhibits enzyme action.



## CLASSIFICATION

The enzymes may be classified in four large groups with subgroups.

**A. Hydrolases**—Bring about hydrolyses; that is, they add water to the substrate and simultaneously decompose it

1. **Carbohydrases**—Split higher carbohydrates into simpler ones

a. **Polysaccharidases**—Change polysaccharides to simpler carbohydrates

b. **Saccharidases**—Split the di- and trisaccharides to monosaccharides

2. **Esterases**—Attack ester linkages

a. **Simple esterases**—Split esters of the ethyl butyrate type, forming ethyl alcohol and butyric acid

b. **Lipases**—Convert fats into fatty acids and glycerol

c. **Phosphatases**—Hydrolyze phosphoric acid esters into their main constituents; thus glycerophosphate is hydrolyzed to glycerol and phosphoric acid

d. **Cholinesterases**—Hydrolyze esters of choline.

3. **Proteases**—Attack the peptide linkage ( $\text{—C—N—}$ ) of proteins



a. **Proteinases**—Capable of splitting linkages usually not adjacent to a terminal group; thus they can break off comparatively large peptide chains

b. **Peptidases**—Split off amino acids from peptides or proteins by attacking terminal peptide linkages

4. **Nucleases**—Hydrolyze nucleic acid to their constituents in several stages, each brought about by a different enzyme

These include nucleinases, nucleotidases, and nucleosidases

5. **Amidases**—Attack carbon-nitrogen linkages, splitting off an amino-containing group

a. **Urease**—Converts urea to ammonium carbonate

b. **Arginase**—Splits the amino acid arginine into ornithine and urea

c. **Nuclein desaminases**—Although these enzymes require water they are not, strictly speaking, hydrolases; they split off  $\text{NH}_2$  from the substrate which becomes oxidized in the process; adenase is one example

**B. Transferases**—Cause the transfer of certain groups from one compound to another

1. **Transaminases**—Effect the transfer of the amino group of certain amino acids to certain  $\alpha$ -keto acids, reversibly

2. **Transacetylases**—Effect the transfer of the acetyl group reversibly

3. **Transphosphorylases**—Move phosphate groups from one organic compound to another

4. **Transpeptidases**—Effect the transfer of amino acids or peptides from one peptide to another

a. **Form peptide linkages** (Require sources of energy)

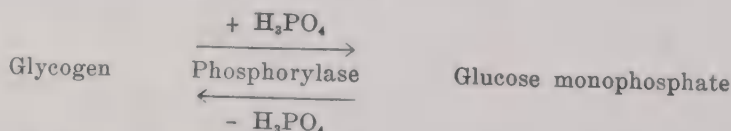
e.g.,  $\text{C}_6\text{H}_5\text{COOH} + \text{glycine} + \text{ATP} \rightarrow \text{hippuric acid} + \text{ADP} + \text{phosphate}$   
(not reversible)

b. **Effect transfer of peptide bonds** (Not much energy required)

e.g.,  $\text{Glutathione} + \text{leucine} \rightleftharpoons \gamma\text{-glutamyl-leucine} + \text{cysteinylglycine}$

5. **Transglycosidases**—Enzymes of carbohydrate metabolism

Include a number of enzymes which add phosphoric acid and simultaneously produce changes in the substrate; for example:

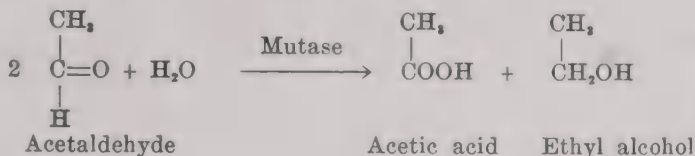


They are quite reversible and responsible for the breakdown and building-up of carbohydrates as well as for energy changes in muscle (see Chapter 16)

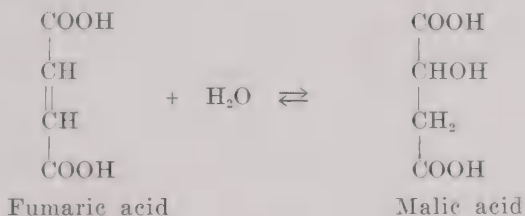
6. **Transmethylases**—Effect the transfer of methyl groups; aid in synthesis of choline, sarcosine, betaine, etc.

C. Oxido-reductases—Enzymes concerned in biological oxidations

1. Dehydrogenases—Remove hydrogen from the substrate, but only if a suitable hydrogen acceptor is present
2. Mutases—Cause the simultaneous oxidation and reduction of two molecules of the same compound; aldehyde mutase is an example:



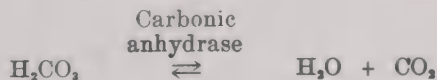
3. Hydrases—Add water to the substrate without hydrolyzing it; an example is fumarase. One may consider the oxygen of the water going to one part of the molecule and the hydrogen atoms to another:



4. Oxidases—Activate the oxygen of molecular oxygen, peroxides, and other compounds
5. Peroxidases—Transfer oxygen from  $\text{H}_2\text{O}_2$  or organic peroxides to the substrate, usually phenols
6. Catalases—Decompose  $\text{H}_2\text{O}_2$ , liberating molecular oxygen (may also act as peroxidases [Tauber, 1952])
7. Other enzymes involved in cell oxidations; there are many of these; some will be considered in Chapter 14

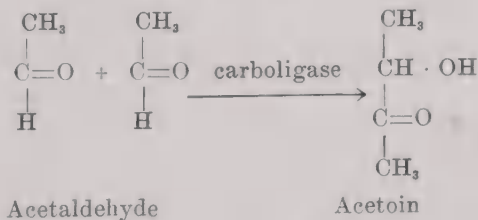
C. Desmolases—Break or form carbon chains

1. Carboxylases—Decompose organic acids with the liberation of carbon dioxide
2. Carbonic anhydrase—Catalyzes the following reaction reversibly:



It is not a true desmolase but it is included here because it resembles the carboxylases.

3. Carboligase—Links carbon chains together:



There are many other types of enzymes which cannot be included in the above classification. In succeeding chapters some of them will be discussed, as will also most of those in the categories given.

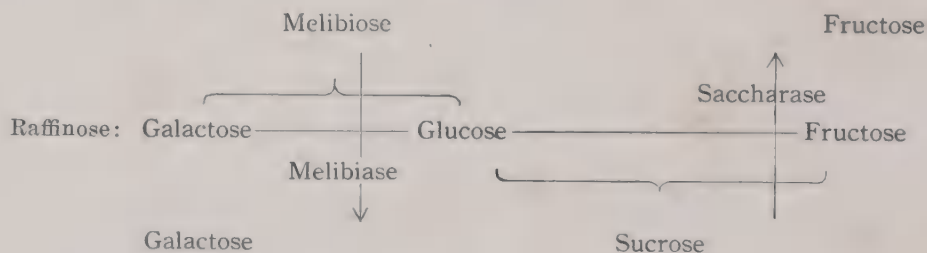
## SPECIFICITY

The enzymes are highly specific; that is, a given enzyme will act on only a certain particular substance or sometimes on closely related substances. This specificity is undoubtedly due to the fact that the enzyme attacks a compound

at a definite type of linkage. Hence there are many apparent exceptions to this property of specificity. Nevertheless, it may be stated quite definitely that broad boundaries are never crossed; proteases do not attack starches, carbohydrases do not split fats, etc. In fact, there are many examples of very narrow specificity. Urease acts only on one substance, urea. As explained in Chapter 3, *maltase* splits alpha-methyl glucoside but not beta-methyl glucoside, while *emulsin* splits beta-methyl glucoside but not the alpha compound. The dipeptidase of intestinal juice will split peptides made up of naturally occurring amino acids, the L-amino acids, but not those containing the D-amino acids. Thus, glycyl-L-alanine will be attacked but not glycyl-D-alanine.

In some cases an enzyme will act upon two or more different substrates with different intensities. Thus pancreatic lipase hydrolyzes aliphatic esters rapidly but cholesterol esters slowly, and emulsin does not split all beta-D-glucosides equally rapidly (Fodor). This might be termed *relative specificity*.

Because of specificity of enzyme action, it is possible to decompose a particular compound in different ways by different enzymes. An example of this is the action of saccharase and of melibiase on the trisaccharide raffinose, which is composed of galactose, glucose, and fructose. The hydrolysis by the enzymes saccharase and melibiase is indicated thus:



Melibiase yields the monosaccharide, galactose, and the disaccharide, sucrose, while saccharase yields the monosaccharide, fructose, and the disaccharide, melibiose.

## FACTORS INFLUENCING ENZYME ACTION

Various factors influence the velocity of enzyme reactions. Among them are the following:

- |                               |  |
|-------------------------------|--|
| 1. Concentration of enzyme    | 4. Temperature                                 |
| 2. Concentration of substrate | 5. Products of reaction                        |
| 3. Hydrogen ion concentration | 6. Effects of light and other physical factors |
|                               | 7. Time  |

**Concentration of Enzyme.**—The velocity of an enzyme reaction is directly proportional to the concentration of the enzyme; that is, the more enzyme, the faster the reaction; if one doubles the amount of enzyme, the rate of reaction is usually doubled. It is particularly true at the beginning of the reaction but may not hold as the reaction continues. This may be due to the presence of impurities which inhibit the reaction (Fig. 26).



**Concentration of Substrate.**—The velocity of an enzyme reaction increases as the concentration of the substrate increases but only up to a certain point and not in strict proportionality. Then the rate continues more or less constant as the substrate continues to be increased (Fig. 27).

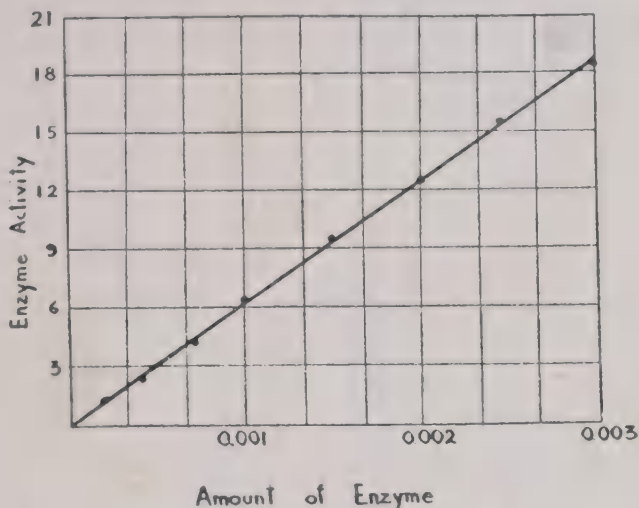


Fig. 26.—The effect of enzyme concentration on enzyme activity. These data were obtained by determining the number of milligrams of reducing sugar formed in digestion mixtures containing different amounts of pancreatic amylase. The pancreatic amylase was supplied as duodenal contents and the values of the abscissa indicate the number of milliliters of duodenal contents present in the digestion mixtures. The digestion mixture was buffered at an optimum pH and contained optimum amounts of chloride and substrate. (From Myers, V. C., and Free, A. H.: *Am. J. Clin. Path.* 13: 42, 1943.)

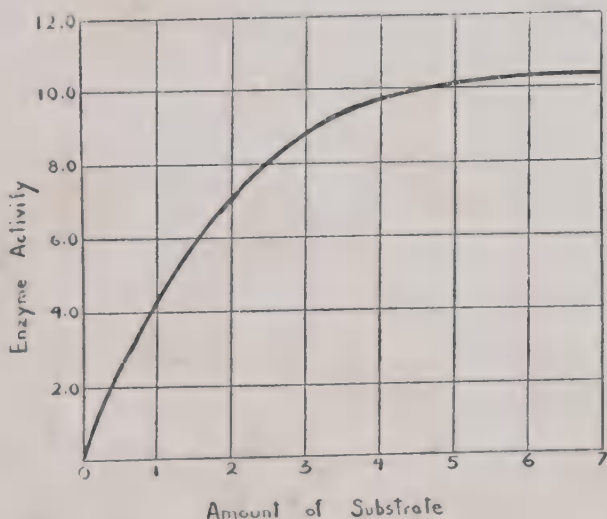


Fig. 27.—The effect of substrate concentration on enzyme activity. These data were obtained by determining the number of milligrams of reducing sugar formed in reaction mixtures in which the amount of enzyme (pancreatic amylase) was constant. The amount of substrate indicates the number of milliliters of 1 per cent soluble starch present in the reaction mixtures all of which had the same total volume. (From Myers, V. C., and Free, A. H.: *Am. J. Clin. Path.* 13: 42, 1943.)

**Hydrogen Ion Concentration.**—Enzyme reactions are influenced by varying the hydrogen ion concentration. The "optimum pH" is that pH at which a certain enzyme will cause a reaction to progress most rapidly. The optimum pH, however, is dependent on various conditions; for example, the kind of

buffer, the particular substrate, and the source of the enzyme may all have an influence. In Table XXIV are illustrated these points, with the optimum pH's for several enzymes under differing conditions (see also Fig. 28).

In Table XXIV is clearly shown, moreover, the wide range of hydrogen ion concentrations at which enzymes are most effective. Most enzymes not only have an optimum pH range, but also tend to be inactivated as the pH changes in either direction beyond that range.

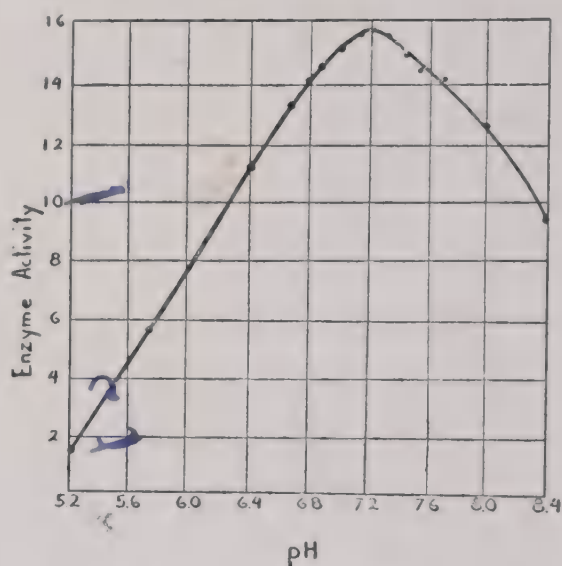


Fig. 28.—The effect of pH on enzyme activity. These data were obtained by determining the number of milligrams of reducing sugar formed in reaction mixtures in which the amount of enzyme (pancreatic amylase) and substrate (soluble starch) was constant. The pH was adjusted by using phosphate buffers ( $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ ) in which the different hydrogen ion concentrations were attained by using different ratios of the acid and alkaline salt. (From Myers, V. C., and Free, A. H.: *Am. J. Clin. Path.* 13: 42, 1943.)

TABLE XXIV

SHOWING THAT THE OPTIMUM pH VARIES WITH THE TYPE OF SUBSTRATE, THE BUFFER, AND THE SOURCE OF ENZYME\*

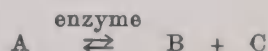
ENZYME	OPTIMUM pH
Amylase, source	
Pancreatic	6.8
Malt	4.4-5.2
Salivary, acetate buffer	5.6
Salivary, phosphate buffer	6.5
Pepsin, substrate	
Egg albumin	1.5
Casein	1.8
Hemoglobin	2.2
Phosphatase, source	
Bone	8.4
Kidney	8.8-9.2
Plant	3.4-6.0
Synthetic action	9.4

\*After Hauber, H.: *The Chemistry and Technology of Enzymes*, New York, 1949, John Wiley & Sons, Inc.

**Influence of Temperature.**—The velocity of an enzyme reaction is increased about twice the initial value with each  $10^\circ \text{C}$ . rise in temperature until the *optimum temperature* is attained. For animal enzymes the optimum

temperature is usually 40-50° C., while for plant enzymes it is higher, usually 50-60° C. Above this, the rate decreases and at a certain point (usually at about 60° C.) the enzyme is destroyed. Dry enzyme preparations withstand heat better than solutions; this harmonizes with the view that enzymes are proteins and heating in the presence of water denatures them.

**Products of Reaction.**—The usual enzyme reaction, *in vitro*, tends to become retarded in rate as it progresses, due to the accumulation of products of reaction. The explanation of this is twofold. In the first place the enzyme reaction is, theoretically at least, a reversible one.

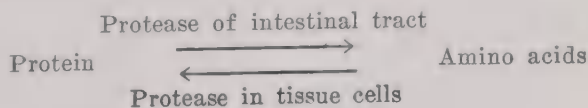


If B and C could be removed as fast as they are formed, the reaction would be 100 per cent complete. However, in the test tube they are not and consequently the reaction is not complete. It is, however, usually more than 90 per cent complete. The chief reason for the retarding action of the products of digestion on the *rate* of the reaction is that some of the reaction products, being structurally similar to the substrate, combine with the enzyme much as the substrate had done. This inactivates some of the enzyme.

**Effects of Light and Other Physical Factors.**—Enzymes may be accelerated or inhibited in their activity by light rays. Red and blue light will increase the action of salivary amylase and of several other enzymes. Ultraviolet rays and radium emanations usually have the opposite action. Violent shaking of enzyme solutions has a destructive effect upon them, due to denaturation of the protein enzyme.

## REVERSIBILITY AND SYNTHETIC ACTION

If enzyme reactions are, as stated previously, reversible, this should be the basis for syntheses in the body. For example, proteins are digested to amino acids by proteases. Amino acids are absorbed from the gastrointestinal canal and are carried by the blood to all the tissues of the body. In all cells we find proteases present. Hence it is deduced that these enzymes should be capable of reversing the proteolytic process and of synthesizing the cell proteins from the amino acids.



The reversibility of many enzyme reactions has been shown. Disaccharides have been synthesized from monosaccharides; esters and fats have been synthesized from their constituents; and a natural tripeptide, glutathione, has been synthesized enzymatically from its amino acid components and also from peptides. (Snoke and Bloch.) The pH for the synthetic reaction sometimes differs from that of the disintegrating one; the concentration of the reactants must be high; and the rate of the reaction is usually very low. Furthermore,



the point of equilibrium frequently is so far to the right as to make it practically impossible to observe any reverse, or synthetic, action.

**Autolysis.**—If fresh tissue is incubated with suitable precautions for sterility, it will disintegrate and may liquefy. The proteins are hydrolyzed by enzymes present that are called *cathepsins*. The process is known as autolysis. Cathepsins are probably present in all animal tissues but are found in higher concentration in liver, spleen, and kidneys. A cathepsin is also present in gastric juice. In the tissues these enzymes are believed to be in an inactive form and during life the pH of the body fluids is unfavorable for their activation as well as for their activity. After death the tissues become more acid because of the liberation of lactic and other acids. This initiates autocatalytic activation, that is, the activation of the proenzyme by minute amounts of the free enzyme, formed presumably by the acid. There is now also a more favorable hydrogen ion concentration for the enzyme and proteolysis occurs. The cathepsins include both proteinases and peptidases with the result that those tissues containing considerable amounts of cathepsins are hydrolyzed to the various split products of the proteins, including the amino acids.

Physiological phenomena which may be explained by catheptic activity are involution of the mammary gland at the end of lactation, the involution of the uterus after parturition, and generalized atrophy of old age. In these a diminished blood supply prevents adequate buffering of acids formed in tissue metabolism. Pathologically, "wasting diseases," such as muscular dystrophy, pathologic degeneration, atrophies, and the resolution of the exudate in lobar pneumonia, have been explained on the same basis. If inhibitory substances are present, autolysis will not occur, of course, and this is thought to be the chief reason why caseous tuberculous material does not autolyze to an appreciable extent.

## INHIBITORS AND ACTIVATORS

**Inhibitors.**—There are many substances, besides the products of reaction, which inhibit enzyme reactions. Such substances, sometimes called enzyme "poisons," fall into several categories. (1) Those which act upon the prosthetic group. CO, HCN, azide, and H<sub>2</sub>S, for example, inhibit enzymes containing an iron-porphyrin complex as the prosthetic group. Oxidizing and reducing agents also may inhibit enzymes, usually by an action on the prosthetic group. Many other substances like NaF, which inactivates enzymes requiring calcium, and diethyldithiocarbamate, which inhibits copper-containing enzymes, effect their action by combination with the prosthetic group. (2) Those which impair the protein fraction of the enzyme. In this group are found the heavy metals. They react with the —SH groups of the protein component of many enzymes and thus destroy their functional activity. One must be careful, for instance, when using urease for urea determinations (see page 515), that the glassware is free from traces of the mercury in Nessler's solution, often used in the same determination, since the mercury will inactivate the urease. Aluminum ions are said to inhibit peptic action. This may be one of the reasons for the benefits derived from aluminum hydroxide therapy in the treatment of peptic ulcer.

) Competitive inhibitors. These are substances which compete with the substrate for the enzyme because they resemble the substrate in chemical structure. Succinic oxidase is inhibited by malonic acid, the structure of which is so similar to that of succinic acid that the enzyme is "tied up" by it. To this group may also be assigned the other "structural analogs" discussed in Chapter 24. These resemble structurally other parts of the enzyme systems. The resemblance is so close that they take their places in such systems but are not capable of competing the reactions. Certain proteins have been shown to be inhibitors of particular enzymes. In soybeans and other legumes there are globulins present which inhibit trypsin. (Ham and Sandstedt). Human blood serum also contains a factor, probably a protein, which is antiproteolytic. Into which of the above categories these would fall is not certain.

Some antiseptics have an inhibitory action, but others have not and are useful in enzyme experimentation. Since enzymes are proteins, they are just as liable to destruction by micro-organisms as other proteins. Consequently, in enzyme experiments it is essential to use an antiseptic which will not inhibit the enzyme. Toluene is a useful one.

In life processes the inhibitors and activators discussed above undoubtedly play important roles. There are, in addition, other factors which must have important effects. It is known that the hormones (see Chapter 23) regulate virtually all life processes, and they must influence enzyme reactions in some way. However, except in a very few instances, no such effect has been demonstrated in test tube experiments.

**Activators.**—Many enzymes are present in the cells or secretions in an inactive state. These are called "zymogens" or "proenzymes." Pepsinogen, trypsinogen, and prorennin are examples and are precursors of pepsin, trypsin, and rennin, respectively. These must be activated before they can catalyze their specific reactions. Some zymogens are autocatalytically activated. In other words, they activate themselves. Thus, pepsinogen in the presence of HCl forms small amounts of pepsin, which then rapidly converts the rest of the pepsinogen to pepsin. Another method of activation is by the action of one enzyme on another, similar to the action of pepsin on its own precursor. Examples of this are the activation of chymotrypsinogen by trypsin and the activation of trypsinogen by the enzyme specific for this purpose, enterokinase. As stated previously, most enzymes require "activators" or "coenzymes" for efficient functioning. In this connection the term "activators" is misleading; "accelerators" would be better. Enzyme activators or accelerators are inorganic and coenzymes, organic.

**Coenzymes.**—The coenzymes are dialyzable, heat-stable organic compounds, necessary for the functioning of enzymes. They are associated with, but usually separable from, the enzymes they activate. The structure and function of several have been elucidated, and they are assuming great importance in biochemistry. One of the coenzymes necessary for yeast activity was found to contain, as its prosthetic group, niacin amide. It is called diphosphopyridine nucleotide (DPN), or coenzyme I (Co I). Another coenzyme has a similar composition, with one more phosphate group. It is triphosphopyridine

nucleotide (TPN), or coenzyme II. Because of the presence of the niacin amide, both of these coenzymes function as hydrogen donors and acceptors and take part in numerous oxidation-reduction reactions. Their formulas are given on page 347. Coenzyme A is a derivative of pantothenic acid and is involved in transacetylation. That is, it forms a union with the acetyl group and is then "acetyl coenzyme A." The acetyl group may then be transferred to another group. Its formula is on page 296.

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## Chapter 10

### DIGESTION

Very few substances are ready for use by the body in the state in which they are ingested. Water, some lipids, some monosaccharides, the vitamins, and organic salts make up almost the entire list of such substances. The rest must be digested before they can be utilized. Digestion is that process whereby various foodstuffs are converted by the body into products suitable for absorption and utilization.

Digestion is, as a rule, aided by cooking. Many of the foodstuffs are partly hydrolyzed by cooking. Collagen and starch are good examples. Some protein foods are rendered more digestible by coagulation. Egg white is one of these, since it has been shown that, contrary to common opinion, raw eggs are not as easily digested as cooked eggs. Most vegetables and fruits are softened by heat. The processes of cooking aid digestion in a physiological manner, because the improved flavors generally brought out reflexly stimulate the flow of the various digestive juices. The heating of food also destroys parasites and bacteria, which, besides producing other deleterious effects, often inhibit digestive and absorptive processes. Cooking destroys any injurious enzymes which may be present. One example is the thiaminase of raw clams, which inactivates vitamin B<sub>1</sub>. The improvement in the nutritive value of raw soybean meal by cooking is well known in the fields of poultry and cattle feeding. The cause of this has been the subject of much study, but it is not clear whether it is due to a change in the structural condition of the amino acids, to the abolition of the effect of the trypsin inhibitor present upon trypsin's digestive activity, or to some specific action of this same trypsin inhibitor upon growth (Klose).

Another aid to digestion is mastication. It is generally agreed that subdivision of the food material hastens digestion and tends toward more complete digestion by presenting a greater surface to the digestive fluids.

The most important factor in digestion, however, is the action of the various enzymes in the alimentary tract. They require suitable conditions for favorable activity and, in most cases, these conditions are found in that tract. The prompt removal of the products of digestion by absorption favors enzyme action since this, theoretically, at any rate, is always a reversible reaction. According to the law of mass action, removal of the products of such a reaction tends to cause it to proceed faster toward the side from which the products are being removed. Consequently good absorption favors digestion, while any factor which hinders absorption has a deleterious effect upon it.

#### **SALIVA**

Saliva is the mixed secretion of the parotid, submaxillary, sublingual, and buccal glands. It contains about 99.4 per cent water and has a specific gravity of 1.002 to 1.008. Some 1,500 ml. is believed to be the approximate daily secre-

tion in man. The secretion of saliva is entirely under the control of the nervous system. A variety of stimuli cause an increased flow by reflex stimulation. This is true whether the stimulus is psychic (the sight, smell, or thought of food), mechanical (as produced by chewing paraffin), or chemical (as caused by the action of acids, salts, etc., on the taste buds). There seems to be no hormonal control of salivary secretion.

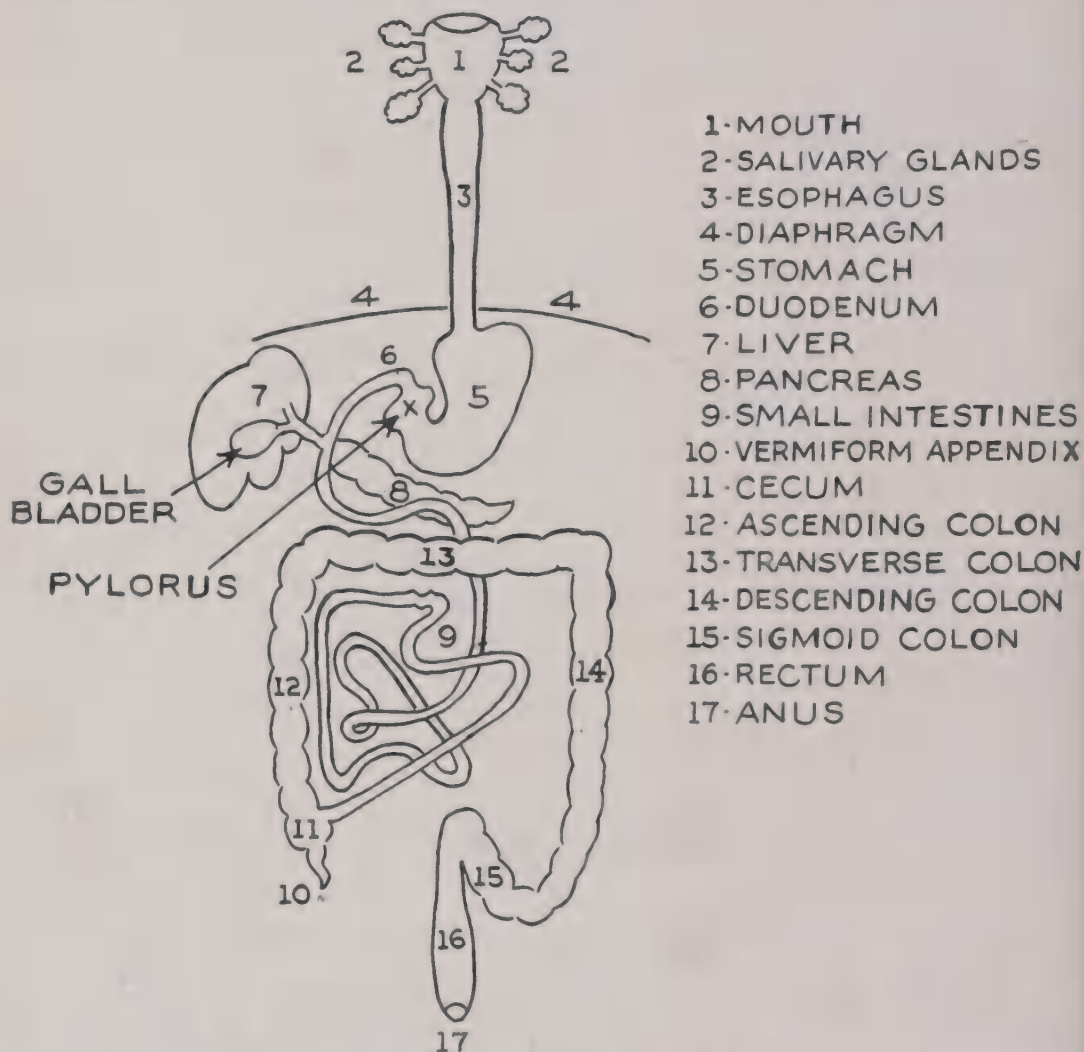


Fig. 29.—Diagram of the alimentary tract. (Modified from Mottram, V. H.: *Physiology*, New York, 1928, W. W. Norton & Co. Inc.)

Saliva is almost colorless and rather viscid, and if a quantity of saliva in a vessel is exposed to air, the surface becomes covered with an incrustation consisting of calcium carbonate with a small proportion of organic matter. The reaction of the saliva of a given individual is not constant. "Resting" saliva is slightly acidic, pH 6.4 to 6.9, while saliva obtained during active stimulation of the glands is neutral to slightly alkaline, pH 7.0 to 7.3.

The solid constituents of saliva comprise albumins, globulins, mucin, enzymes, urea, uric acid, and inorganic salts. The inorganic components differ markedly in concentration from those of blood serum, but the nonprotein nitrog-

nous constituents (urea, uric acid,  $\text{NH}_4$  salts) appear to bear some relationship to these same constituents in the blood. Amino acids are absent and glucose occurs in extremely small amounts in the saliva of healthy individuals (11-30 mg. per 100 ml.) (Young). The salivary glands, therefore, appear to be quite selective in their secretory action.

The chief inorganic ions present are  $\text{K}^+$ ,  $\text{PO}_4^-$  and  $\text{Cl}^-$ , with smaller amounts of  $\text{Na}^+$ ,  $\text{Ca}^{++}$ , and  $\text{SO}_4^-$ . Some of these may combine to form insoluble precipitates. This may be aided by changes in the pH brought about by decomposing food material left between the teeth or by evaporation of  $\text{CO}_2$ , held in solution in the saliva, as soon as it meets atmospheric conditions. Thus "tartar" may be formed. This consists chiefly of calcium carbonate and phosphate. Salivary calculi sometimes are formed in the ducts and are similar in composition to tartar; namely,  $\text{Ca}_3(\text{PO}_4)_2$  or  $\text{CaCO}_3$ . It is usually stated that a clump of bacteria or a foreign body, such as a blood clot, establishes a nucleus around which the precipitation of these salts occurs. However, calcium oxalate may be the precipitated salt, which, together with mucin and globulin, may form the calculus. Increased acidity is necessary for oxalate calculus formation.

### Functions of Saliva

Saliva has a digestive function due to the enzymes present, but it also has other functions. It moistens and lubricates the food, permitting it to be swallowed easily. Saliva holds the taste-producing substances in solution, and so brings them in contact with the taste buds. It dilutes salts, acids, etc., thereby protecting the mucosa. It also has a cleansing action on the teeth, gums, and buccal mucosa. It owes its viscous and lubricating property to its content of mucin. This glycoprotein is present as an alkaline salt, which is soluble at the pH of saliva, but is precipitated on acidification. It is one of the chief buffers present in saliva. Some authorities maintain that saliva has an excretory function, since certain elements and drugs are found in it after administration. Among these are mercury, lead, and potassium iodide. Any part of these lost in expectoration could be considered excreted, but some of the part swallowed may be reabsorbed. Hence it is difficult to see how this can be called true excretion. The same is true of the traces of urea, uric acid, and ammonium salts ordinarily found in saliva.

### Enzymes of Saliva

The principal enzyme of human saliva is an amylase, *ptyalin*. There are also present a maltase, a catalase, a lipase, a urease, and a protease, but these are all unimportant. The saliva of the lower animals is not comparable with that of man, since the same enzymes may not be present. No amylase is found in the saliva of the sheep, goat, dog, or cat.

**Salivary Amylase.**—The amount of this enzyme present in the saliva of the infant is very small until the fourth or fifth month of life. At the age of 1 year the amylolytic power is about as great as in the adult. In old age it again becomes much weaker.



The optimum reaction is pH 5.6 to 6.5, depending upon the substrate, but it can act over a range of pH 4 to 9. As previously stated, the reaction of saliva is from pH 6.4 to 7.3. It is evident that saliva, as secreted, has a hydrogen ion concentration suitable, and frequently optimal, for the activity of its chief enzyme. It is not until a pH of less than 4 is reached that the amylase is inactivated. As will be seen, this occurs in the stomach where the acid gastric juice is secreted. However, salivary digestion may proceed in the stomach for from fifteen to twenty minutes after the saliva-impregnated food is swallowed. The time depends upon the rate of secretion of gastric juice, the type of food present, and the buffering action of the mucus present in both the saliva and gastric juice. Once the acidity has reached pH 4.0 or thereabouts, salivary digestion stops.

The action of salivary amylase is entirely on the polysaccharides, starch and glycogen, and their derivatives. For its action it requires the presence of certain negative ions as activators. Chlorides and bromides are most effective, with iodides and nitrates next in value and sulfates, phosphates, and acetates still less effective. It converts starch to soluble starch, and this, through the series of dextrans, to maltose. Maltose is split off all along the line. Finally, a small proportion of the maltose may be hydrolyzed to glucose by the maltase present. This, however, occurs only to a very slight extent.

## GASTRIC DIGESTION

Gastric juice consists of water (99.4 per cent), HCl, mucins, and the enzymes pepsin and lipase. The hydrochloric acid is secreted by the parietal cells and the pepsin by the chief cells. According to Glass and Boyd, the gastric mucous substances comprise (1) the mucoid of the visible gastric mucus, secreted by the surface epithelium, (2) dissolved "mucoproteose," a digestion product of the visible gastric mucus, and (3) "glandular mucoprotein," secreted by the neck mucous cells of the gastric glands. "Glandular mucoprotein" is considered by these authors to be the main carrier of the "intrinsic factor" of the human gastric juice. (See page 196.)

**Early Experiments on Gastric Digestion.**—Reaumur (1683-1757) experimented chiefly on a bird, a kite. He caused the bird to swallow perforated metallic containers of food and discovered that the food was dissolved out. The same type of experiments was repeated and extended by Abbé Spallanzani (1729-1799). Using himself as the experimental animal, he swallowed sponges attached to strings, withdrawing them and squeezing out the gastric juice, and demonstrated the solvent power of gastric juice outside the body. He also discovered that it prevents putrefaction, but he failed to recognize the acid character of this fluid. Carminati, at about the same time, declared that it was not acid after fasting but became acid after partaking of food. Werner, in 1800, and others confirmed Carminati's observation, but, in 1812, Montegre, who was able to vomit whenever he wished, declared that gastric juice contained no acid, was not a food solvent, and was probably only swallowed saliva. In 1824, an English scientist, Prout, proved gastric juice to be acid and that the acidity was due to HCl. This was independently discovered by Tiedemann and Gmelin. It is evident that knowledge of gastric physiology was chaotic at the beginning of the nineteenth century. In fact, Magendie, who was one of the leading physiologists of that time, stated in his textbook that gastric juice was without digestive power outside the body, and the general opinion was that any digestion taking place in the stomach was due to mechanical, rather

an to chemical, action. It was an American Army surgeon, Beaumont, who brought order out of chaos in this field. In 1822 an accidental discharge of a shotgun near the upper abdomen of a young French-Canadian, Alexis St. Martin, resulted in a permanent gastric fistula. For many years Beaumont was able to make observations and to obtain human gastric juice through this opening. He confirmed the facts of the acidity of gastric juice and its solvent power and studied the temperature, movements, and appearance of the interior of the stomach. Among his numerous physiological observations was the fact that the presence of certain foods in the stomach stimulate secretion and that intense emotions inhibit it. He studied the relative digestibility of different common foods, and nutritionists agree that his conclusions are thoroughly sound. In fact, there are very few of his observations which have not stood the test of time, and they have become the foundation of modern gastric physiology.

Beaumont maintained that there must be some "principle" present with digestive activity. In 1836 the actual discovery of pepsin was made by Schwann, who showed that boiled gastric juice, although still acid, had no digestive power. He gave this active principle the name pepsin, from a Greek word meaning digestion.

Later, Heidenhain devised and, more recently, Pavlov improved methods for producing accessory stomachs in experimental animals. These involve detaching a flap from the stomach, inverting this in such a way that it forms a pouch with an opening through the body wall and skin of the animal. Blood and nerve supply must be unharmed, and consequently a flow of pure gastric juice, uncontaminated by food, and under the same nervous and vascular conditions as the main stomach, is obtained. Using such "Pavlov pouch" dogs, scientists have made many contributions to the physiology and biochemistry of gastric digestion.

### Gastric Juice

The secretion of gastric juice is said to be continuous in man but this is not certain. It is intermittent in animals in which experimental conditions are carefully controlled. If it is continuous in man, it is probably at an extremely slow rate. There are three types of stimuli which increase the flow of gastric juice:

- (1) *Cephalic phase of secretion.* Psychic stimuli have long been known to have such an effect. The thought, smell, or taste of food or even an action related to food (the conditioned reflex of Pavlov, such as the ringing of a dinner bell) all reflexly cause an increased flow of gastric juice. The production of low blood sugar in man by the injection of insulin is followed by an increased secretion of gastric juice, rich in both HCl and pepsin. The low blood sugar is believed to be the stimulus for the parietal cell, brought about by a central stimulation of the vagus. (2) *Gastric phase.* When food is present in the stomach, gastric juice continues to be secreted longer than would be expected from psychic stimuli alone. Beaumont declared that mechanical stimulation of the mucosa would cause secretion. This was denied by Pavlov, but in 1925 Ivy and associates showed that it is true. Application of a distending force will produce a flow of gastric juice after some time. Certain foods and, indeed, specific constituents of the foods, are powerful stimulants; for example, meat extracts and the products of protein digestion, the polypeptides (so-called proteoses and peptones). These "secretagogues" probably act indirectly; that is, in some way they cause the formation of a hormone in the pyloric mucosa that is absorbed into the blood stream, carried back to the gastric glands and stimulates them to secrete. This hormone, discovered by Edkins, is called *gastrin*. He found that when the pyloric mucosa was ground up and extracted with "peptones," or other of the stimulating substances, a fluid



was obtained which, on intravenous injection, had a powerful secretory effect. Histamine has a similar secretory effect, but the juice produced is high in acid and low in pepsin. However, histamine-free gastrin has been obtained by Komarov from an HCl extract of pyloric mucosa, and further evidence for the existence of gastrin has been furnished by Grossman and co-workers. Dilute ethyl alcohol also stimulates gastric secretion and is often used as a test meal. It is possible that alcohol produces its action by liberating histamine. (3) *Intestinal phase*. When the products of gastric digestion leave the stomach and enter the duodenum, they have a stimulating effect upon gastric secretion. The mechanism of this action is not at all clear, but it is probably due to substances present in the foods. These are absorbed and, perhaps, stimulate nerve endings.

**Inhibitory Influences.**—The activity of the gastric glands may be inhibited by depression of the formation of secretagogues. This may occur during the psychic phase, as well as during the gastric and intestinal phases whenever digestion or propulsion of food is impaired. Fat has a definite inhibitory effect upon gastric secretion. The common belief that greasy foods are “hard to digest” rests upon a solid foundation. All three secretory phases, cephalic, gastric, and intestinal, seem to be similarly depressed, as is also the motility of the stomach. The quantity, acidity, and enzymic potency of gastric juice are all reduced, but the mechanism is rather obscure. It has been shown that the fat causes the production of an inhibitory hormone, enterogastrone, in the intestinal mucosa. Enterogastrone has been purified sufficiently to be tested on human beings in certain pathological conditions. It is believed to be a mixture of a secretion inhibitor and a motility inhibitor, which can be separated from each other. Another inhibiting effect is that of the hydrochloric acid secreted. This is termed “acid inhibition.” When the gastric contents reach a certain threshold, perhaps 0.03 N in human beings, secretion begins to slow up, and at about 0.10 N the acid-forming cells are almost completely inhibited. The intestinal phase of gastric secretion is similarly affected. Acid inhibition is probably brought about also by the enterogastrone mechanism.

Inhibitors of carbonic anhydrase have also been shown to have a depressant effect upon the secretion of gastric HCl. This enzyme may play some role in the formation of HCl by the gastric mucosa and its inhibition would be expected to decrease the secretion of HCl. (Davies and Edelman, 1951; Janowitz.)

### Hydrochloric Acid

The secretion of a strong mineral acid by the gastric mucosa is almost unique from a biological standpoint. At the instant of secretion by the parietal cells it has a concentration of about 0.17 N and a pH of 0.87. Pure parietal secretion apparently contains no phosphate, neutral chloride, or combined acid. It is practically free from everything except HCl and is approximately isotonic with blood plasma and the body fluids. According to Hollander, it is of remarkably constant composition. How, then, does the parietal cell manufacture such a strong acid from fluids, such as blood plasma and tissue



id, which are neutral or slightly alkaline (pH 7.3)? There have been a number of theories to account for this phenomenon, but only one will be outlined here. It has recently been formulated by Hollander, and it takes into account the fact that the parietal cells contain a high concentration of carbonic anhydrase, which catalyzes the following reaction:



This is a reaction, which, as is well known, proceeds in either direction by itself, but the enzyme hastens it enormously. The scheme in Fig. 30 illustrates this hypothesis.

### CHEMISTRY OF HCl FORMATION BY THE PARIETAL CELL

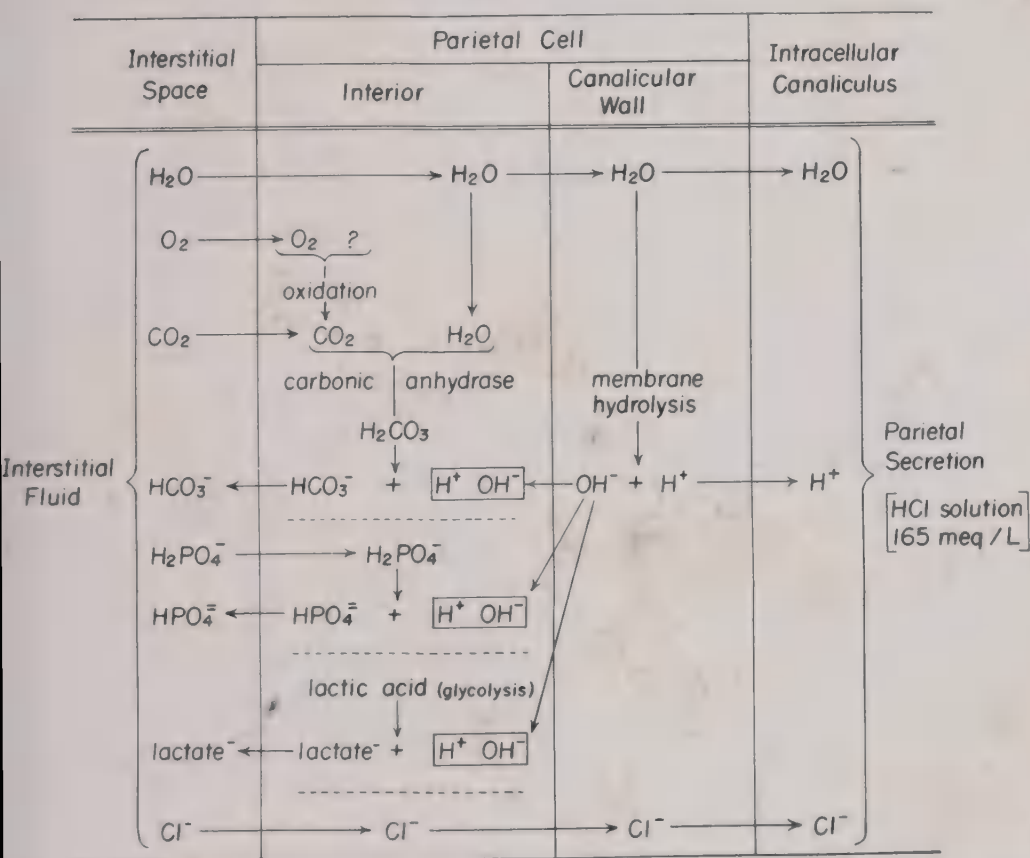


Fig. 30.—Chemistry of HCl formation by the parietal cell. (Courtesy of Dr. Franklin Hollander.)

Since uncontaminated parietal secretion is entirely devoid of cations other than  $\text{H}^+$ , and of anions other than  $\text{Cl}^-$ , it is assumed that the membrane of the intracellular canaliculus is permeable only to these ions and to water. On the other hand, the cell membrane separating the parietal cell from the interstitial fluid must be permeable to a variety of ions, including  $\text{Na}^+$ ,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{lactate}^-$ , and to  $\text{CO}_2$  and  $\text{O}_2$ . The actual formation of  $\text{H}^+$  ions occurs in the canalicular wall by the hydrolysis of water:



This reaction proceeds to the right because of the selective permeability of the membrane, whereby  $H^+$  ions are passed into the gastric juice along with  $Cl^-$  ions, and because the  $OH^-$  ions may immediately be combined with the  $H^+$  ions formed by the dissociation of the  $H_2CO_3$ . The formation of this acid is accelerated by the carbonic anhydrase in the cytoplasm of the parietal cell. The  $CO_2$ , which gives rise to it (on combination with water), is derived from  $CO_2$  diffusing into the cell or resulting from oxidation due to metabolic processes. Phosphates and lactates also yield  $H^+$  to neutralize the  $OH^-$  formed. If not buffered, this base might well injure the tissue and be the cause of ulcers. (Davies and Edelman, 1951.)

It is to be noted that the blood plasma ultimately receives alkaline factors during or after acid gastric secretion. This harmonizes with analyses of the blood at such times and also with the fact that usually soon after meals the urine secreted is alkaline. This so-called "alkaline tide" is one of the mechanisms for keeping the hydrogen ion concentration of the blood quite constant.

**Functions of HCl and Factors Decreasing Its Strength.**—The HCl of the gastric juice provides a favorable pH for the activity of pepsin. Besides this most important action, it serves other purposes. It has some physical action on the proteins, swelling some and making them more easily digested. It has a slight hydrolytic action, perhaps more on the disaccharides than on other foodstuffs, but even here it is not of great significance. Another action of HCl is to convert the colloidal  $Fe(OH)_3$ , found in some foodstuffs, into monomolecularly dispersed ferric ions. Then these and any other ferric ions present are more readily reduced to ferrous ions at pH 5 or lower by ascorbic acid, cysteine, or the  $-SH$  of proteins, which may be in the food (Granick). The strong acid also has a strong antiseptic action. Alvarez says, "Contrary to popular belief, there is rarely any fermentation in the stomach. Its contents are too acid and the food does not remain long enough for gases to be formed. . . ." If the gastric contents were sufficiently acid at all times, infection could never enter the body by the gastrointestinal route. Undoubtedly a great many infections are prevented by this means, but the gastric acidity is decreased by various factors. These include:

1. Variations in the rate of parietal secretion (the composition is constant but the rate may vary)
2. Dilution by the secretions of the other cells, especially mucus
3. Dilution and buffering by food
4. Dilution and buffering by the saliva that is swallowed
5. Regurgitation of duodenal fluid and bile
6. Dilution and neutralization by a distinct "dilution secretion"

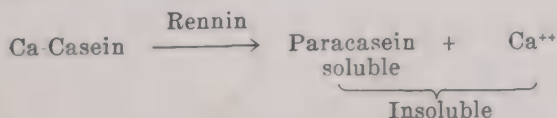
The "dilution secretion" may be formed by the cuboidal cells. It is possibly produced in order to dilute the stomach contents to proper concentration and consistency. The secretion of mucus is undoubtedly of great value. It has a high buffering power and must aid in slowing up the acidification of stomach contents.

## Enzymes

Pepsin, a powerful proteinase, is present in the chief cells as the zymogen, pepsinogen. This is inactive. It has been crystallized by Herriott. The conversion of pepsinogen to pepsin is autocatalytic at pH 4.65 or below; that is, in acid solution a minute amount of pepsin is all that is necessary to be produced from pepsinogen, and then the pepsin converts the pepsinogen rapidly to pepsin. The fact that pepsin exists in the inactive form can be readily demonstrated, since pepsin is more sensitive to alkali than pepsinogen. A neutral extract of gastric mucosa is divided in two parts. Part A is acidified and can be shown to digest protein; it contains pepsin. Part B is treated with an equal amount of water; it will not digest protein in neutral solution and presumably contains pepsinogen. Both are now made alkaline (pH 8.3); then they are neutralized and acidified to pH 2 or less. Part A will now be found to be incapable of digesting protein, while part B, the pepsinogen, has been unaffected by the alkali, and now has proteolytic power.

**Action of Pepsin.**—Pepsin, in acid solution, attacks nearly all native proteins. Exceptions are the keratins, silk fibroin, the mucins and mucoids, and the protamins. Pepsin attacks definite types of peptide linkages, and since most of these happen to be centrally located in the various protein molecules, the split products are, in general, rather large polypeptide chains. Linkages known to be split by pepsin are those peptide bonds joining the amino group of phenylalanine or tyrosine to another amino acid. (Fruton and Bergmann.) Probably other linkages are also broken by pepsin, since gelatin, which is readily digested by pepsin, has practically no tyrosine and very little phenylalanine in its molecule. It is usually said that pepsin splits the proteins to "proteoses" and "peptones," but some free amino acids are detached, although not to a very great extent. It has been shown that pepsin can split short chain peptides if the characteristic peptide linkage demanded by pepsin is present. In a general way, then, pepsin digests the proteins into relatively large digestive products, with the release of smaller amounts of short chain peptides and free amino acids, notably tyrosine and phenylalanine. The optimum pH of peptic activity varies from 1.5 to 2.2, depending upon the substrate.

Some textbooks include rennin, another proteolytic enzyme, among gastric enzymes, but this is incorrect. Rennin occurs in the fourth stomach of the calf and, probably, of other young ruminants. Its action is to "clot" milk. It does this by a slight digestive action upon casein. "Paracasein" is produced and this, in the presence of  $\text{Ca}^{++}$ , precipitates as calcium paracaseinate.



Pepsin will effect the same reaction at an early stage of its digestion of casein, and particularly at a pH of about 5.0. Other proteinases are similarly active. Before these facts were known there was a controversy as to whether rennin really existed. Was not the rennetic action simply one phase of peptic action?



However, it has been shown that the two enzymes have quite different isoelectric points, optimum pH's, composition, and other properties. It was then assumed that *both* enzymes were present in human gastric juice, but it has been quite definitely proved that there is no rennin in adult human gastric juice. There would seem to be no need for rennin since casein is precipitated both by pepsin and by HCl and is thus held in the stomach for further digestive action. (Tauber; Dotti and Kleiner.)

When milk alone is ingested by young children, there is only a moderate fall in pH, not sufficient for optimal proteolysis. In fact, only minimal proteolysis occurs under these conditions in the stomach, and much of the clotted milk remains here only a relatively short time. The main burden of protein digestion is shifted to the enzymes secreted into the small intestine. (Wolman.)

**Gastric Lipase.**—The lipase of gastric juice is of little importance because of unfavorable hydrogen ion concentration. The optimum pH is about 7.8 (Ito and Kamisasanuki) and conditions in the stomach are not ideal for its activity. The effect of this enzyme is to split the fats to fatty acids and glycerides. The statement is frequently made that gastric lipase acts only upon emulsified fats. Unemulsified fat would not be easily emulsified in the acid gastric medium and therefore would not be split readily by a weak enzyme. It is evident that the enzyme would be more effective on preformed emulsions such as milk, cream, and egg yolk. This enzyme is obviously unsuited to the acid gastric medium and is of little importance as a digestive agent. Indeed, some authorities are of the opinion that "gastric lipase" is not secreted by the gastric mucosa but is derived from duodenal regurgitation or cellular breakdown.

**Gastric Analysis.**—The clinical procedures for testing various gastric functions include the administration of a test meal, or the injection of histamine or insulin, and the withdrawal by a stomach tube of the gastric contents for analysis. The most widely used test meal is the Ewald meal, consisting of two pieces of dry toast, or the equivalent in soda biscuits, or one shredded wheat biscuit and 250 ml. of water or weak tea. The Boas meal consists of thin oatmeal gruel. Others include meat and potatoes, and the most popular recent "meal" is the Ehrmann alcohol meal which consists of 50 ml. of 7 per cent ethyl alcohol. The meal is given on an empty stomach and is removed either after one hour or at regular intervals. The latter is the Rehfuess fractional system. The stomach tube is left in place, and at fifteen-minute intervals a small sample is removed for analysis or the entire contents are removed, an aliquot portion reserved for analysis, and the remainder returned to the stomach. The single analysis at the end of one hour often gives erroneous data, since the curve of secretory activity cannot be shown by one sample (Fig. 31). The material obtained is strained or filtered and examined both qualitatively and quantitatively.

Qualitative tests include those for butyric acid, lactic acid, occult blood, bile, and, perhaps, trypsin. The presence of the first two acids would point to the presence of yeasts or other microorganisms, and, hence, a lack of free HCl. If blood is present, ulcers, hemorrhages, or other pathological states would be indicated. In testing for blood, a meatless test meal is imperative. Either

le or trypsin is evidence of regurgitation of intestinal contents; this is a frequent normal occurrence. A microscopic examination is also usually made.

The quantitative procedures are gradually being changed to conform to modern chemical ideas. The total acidity comprises the acidity contributed by  $\text{HCl}$ , organic acids, and acid salts, neutralized or buffered by various constituents of the gastric juice and the foodstuffs. The indicators formerly used do not give satisfactory end points. However, if one titrates with a mixture of two indicators, bromphenol blue and phenol red, better results are obtained. Titration proceeds until a color change corresponding to pH 3.6 is attained. This gives the value for free acidity. The titration is then continued until pH 7.0 is reached, which furnishes the total acidity value. The difference between the two figures gives the value for combined acidity. For accuracy in determining the exact shade of color corresponding to the desired pH in each case, buffer solutions are made up of pH 3.6 and 7.0 (using buffer tablets available commercially), containing the same indicator mixture (Hollander). The difference between the total

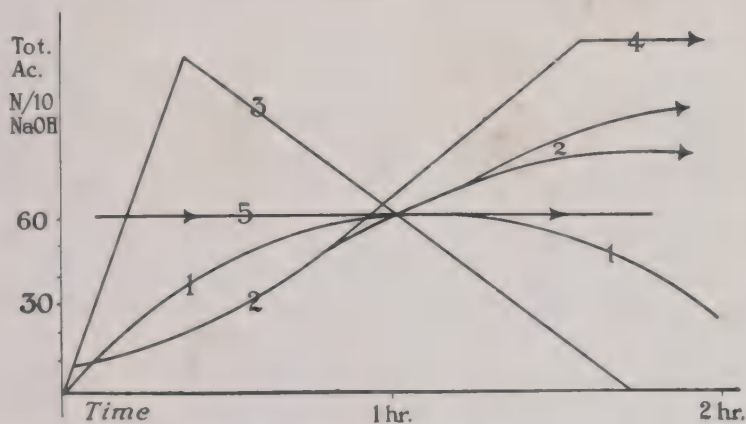


Fig. 31.—Normal and pathological curves after an Ewald meal by the Rehfuess fractional method. 1, Normal curve; 2, delayed digestion with late hyperacidity; 3, larval hyperacidity; 4, marked continued secretion from obstruction. It will be noted that only the one-hour sample had been taken, the same result would have been obtained in all cases. (From Hawk, P. B., and Bergeim, O.: *Practical Physiological Chemistry*, ed. 11, Philadelphia, 1937, P. Blakiston's Son & Co., Inc.)

acidity and free acidity has been assumed to be “combined acidity” (plus acidity due to organic acids and acid salts). However, this would only be true if the titration would terminate at the isoelectric point of the proteins present. Further titration produces alkaline salts of the proteins. Therefore, this value of “combined acidity” is rather a measure of the buffering power of the gastric contents and depends largely upon the amount of protein present. From a clinical standpoint determination of pH or titration of free acid is probably sufficient.

It is obvious that normal values will vary according to the test meal as well as to the methods of analysis used. The clinician usually wishes to ascertain whether there is hyper- or hypoacidity, high or low pepsin, large or small volume secreted, as well as whether abnormal constituents are present. The absence of hydrochloric acid is termed *achlorhydria*. True achlorhydria may occur in pernicious anemia, gastric carcinoma, and in a number of other conditions. A “false” achlorhydria is observed when the hydrochloric acid has



been combined with the protein of the test meal or has been partly neutralized by regurgitated duodenal contents. The latter will have a normal or high total acidity with a low free acidity, while true achlorhydria will have both low total and low free acidity. If the HCl is not entirely absent, but is below normal, the condition is called *hypoacidity*. Hypoacidity frequently accompanies gastric carcinoma, as well as many gastrointestinal ailments, such as gastritis and constipation, secondary anemias, and chronic debilitatory diseases. Many normal pregnant women have low gastric acidities. Of the conditions in which the acidity is elevated (*hyperacidity*), perhaps the most noteworthy are duodenal ulcer and gall bladder disease. It should be emphasized that the acidity can never exceed a certain value (pH 0.87), since the parietal cells secrete a fluid of constant composition.

**Estimation of Pepsin.**—There are a number of methods of measuring the amount of pepsin in gastric contents. The most accurate methods are not adapted to clinical use, and great accuracy is not needed. The classical procedure is that of Mett. In this method, small glass tubes are filled with egg albumin and then boiled to coagulate the protein. They are then placed in definite amounts of the gastric fluid for a number of hours, and the length of the columns of digested protein, which can easily be seen because the columns become transparent, is measured in millimeters. A simple calculation gives the amount of pepsin. A quicker and easier method is based on the milk-clotting power of pepsin. Cow's milk diluted with a buffer of pH 5.0 is used as a substrate. The gastric fluid is diluted in a definite way and the smallest amount of this which will clot 10 ml. of buffered milk in ten minutes is determined. The calculation of the number of clinical units present is very simple. In patients having peptic ulcers there is usually a high pepsin value, while in pernicious anemia, cirrhosis of the liver and various chronic gastric ailments a low pepsin content is found. (Barowsky; Tauber and Kleiner.)

## DIGESTION IN THE SMALL INTESTINE

Digestion in the small intestine is subject to many influences acting simultaneously or in rapid succession. These are both physiological and chemical in nature and may have both physiological and chemical effects. The digested food material leaving the stomach, the "chyme," is acid in reaction. Quite naturally it influences the hydrogen ion concentration of the duodenal contents, which are also subject to the alkaline influence of the bile, pancreatic juice, and the intestinal secretions. These are thrown into the tract at nearly the same site one after the other. The acid also has a role in causing the pancreas to begin its secretory action. Bayliss and Starling found that an HCl extract of intestinal mucosa, upon intravenous injection, accelerated pancreatic secretion. They claimed that an inactive substance in the mucosa was converted to an active one by HCl. The first was termed "prosecretin" and the second, "secretin," but it is now rather definitely established that secretin occurs in the mucosa preformed and in some way is liberated by the action of the HCl of the chyme. It is absorbed into the circulation and carried to the pancreas, where it stimulates the secretion of pancreatic juice. Secretin appears to be a long-chain polypeptide of basic reaction. Another hormone, pancreozymin, causes



stimulation of the secretion of enzymes by the pancreas. It is found only in the upper intestinal mucosa, whereas secretin is found also in the gastric mucosa. Pancreatic secretion is under nervous control also, the impulses coming by way of the vagus nerves.

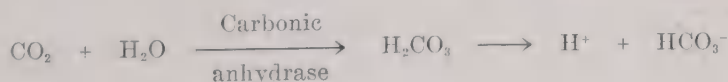
### Reaction of the Intestine

The reaction of intestinal contents will vary considerably for reasons mentioned, as well as for others. In the nursing infant, according to McClendon, the duodenal content is more acid than the average gastric acidity. This is due to the rapidity of the emptying of the stomach. Undoubtedly peptic digestion continues in the intestine. However, in the adult the mechanisms for taking care of the high acidity of the gastric chyme are very efficient. The three secretions poured into the duodenum all have high buffering qualities, the pancreatic juice particularly. As a consequence, the high acidity is rapidly reduced; the lower duodenal contents of the human being ordinarily have an acidity ranging from pH 4.5 to 5.1, and when the ileum is reached the pH is from 5.9 to 6.5.

Mann and Bollman found in experiments on animals that different foodstuffs have specific effects on the reaction of the duodenal contents. Protein produces the most acid, carbohydrate next, and fat least. This action of fat provides a more suitable medium for its digestion. It is suggested that interference with the neutralizing mechanisms, coupled with high protein intake, might be of importance in causing ulcers of the gastrointestinal tract. Mechanical factors, such as the direction of the flow of chyme, seem to determine the site of ulcer. Recent work indicates that both the pepsin and the HCl of the gastric juice are causative factors in ulcer formation, whereas formerly high acidity alone was held responsible.

### Pancreatic Juice

The pancreatic duct joins with the common bile duct to form the ampulla of Vater; thus pancreatic juice and the bile empty into the duodenum at the same point. The total volume of pancreatic juice secreted daily has been estimated at about 500 ml., but this is little more than a guess. The solids present amount to about 1.3 to 1.4 per cent; the specific gravity is about 1.007; and as said before, the fluid is alkaline, with a pH of about 8. The alkalinity is due to  $\text{NaHCO}_3$ . Since carbonic anhydrase is present in pancreatic tissue, and since the administration of an inhibitor of this enzyme has been shown to decrease the bicarbonate content of pancreatic juice, it is probable that most of the  $\text{HCO}_3^-$  is produced according to the following reactions (Hollander):

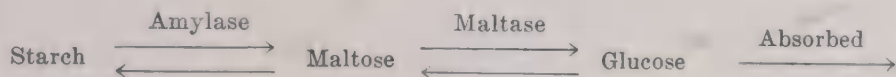


secreted it contains several powerful enzymes, at least two of which are present as the zymogens.

Two proteinases present are trypsin and chymotrypsin. They are in the zymogen form, trypsinogen and chymotrypsinogen, respectively. Trypsinogen is changed into trypsin by enterokinase secreted by the intestinal mucosa. This agent, sometimes termed an activator of the enzyme, has now been definitely shown by Kunitz to be an enzyme. Similarly, chymotrypsinogen is transformed

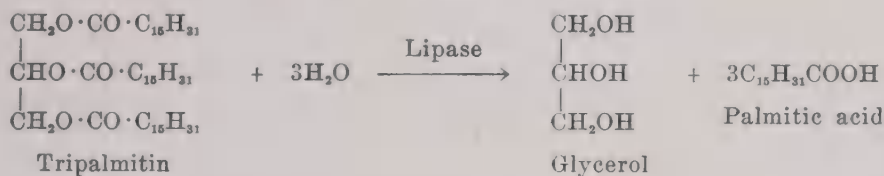


from human salivary amylase. Its action is aided by the removal of the end product maltose. This occurs as the maltase of the intestinal juice catalyzes the hydrolysis of this disaccharide to glucose, which in turn is hastened by the absorption of glucose.

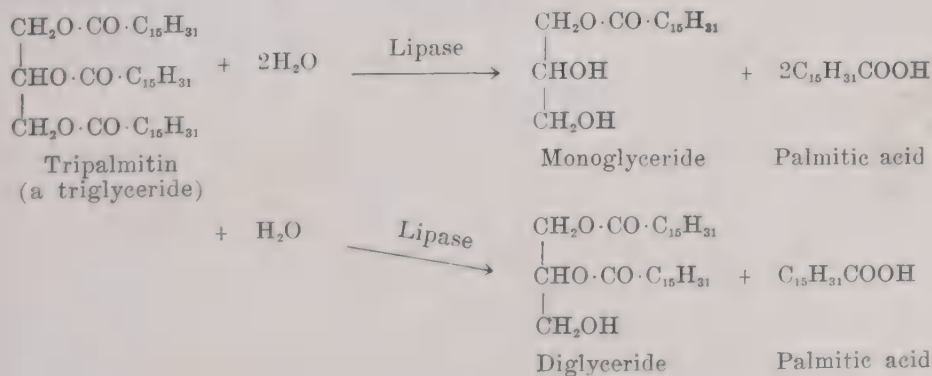


Pancreatic juice itself appears to contain no maltase, but it may contain two other saccharidases, namely, lactase and sucrase, which hydrolyze lactose and sucrose, respectively, to their monosaccharide constituents.

There is present in pancreatic juice a fat-digesting enzyme, steapsin, which is relatively weak as secreted. Whether the lipase is present as a zymogen or not is not definitely known. At any rate its action is tremendously accelerated (or the zymogen is activated) in slightly alkaline solution by a number of different types of substances—calcium salts, soaps, albumin, certain peptides, and, notably, bile salts. Its action is to hydrolyze fats to free fatty acids and glycerol at an optimum pH of about 7.



According to Frazer and Sammons, the above reaction is not completed by lipase under conditions similar to those prevailing in the duodenum. During five hours of in vitro digestion of olive oil by pancreatic lipase, no free glycerol could be demonstrated. Lower glycerides and fatty acids were formed. Similarly, material recovered from the intestines of rats, fed olive oil, contained fatty acids and lower glycerides. This harmonizes with the finding of the Frazer group that an emulsifying system for fat, which is effective under physiological conditions (and efficient emulsification is a prerequisite for fat digestion), is the simple combination fatty acid-bile salt-lower glyceride. The formation of lower glycerides, that is, mono- and diglycerides is as follows:



There is also an esterase present which splits esters composed of monohydric alcohols and monocarboxylic acids.

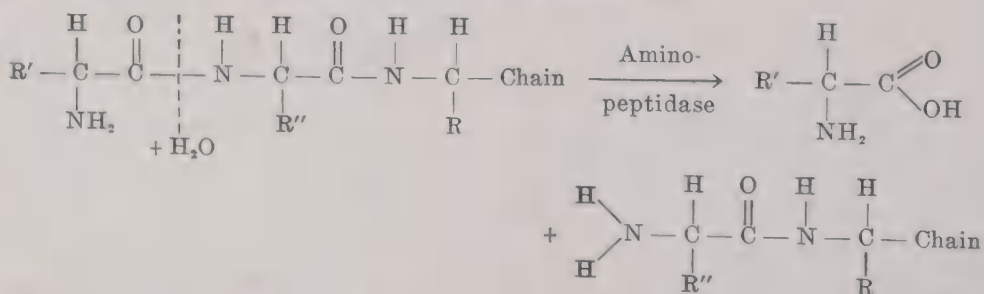


In the pancreatic juice of man and other animals there is found an alkaline phosphatase which hydrolyzes organic phosphates. (Grossman.) "Pancreatin," which is the dried extract of hog pancreas, contains most of these as well as certain other enzymes. Among the latter is a cholesterol esterase which hydrolyzes cholesterol esters, and also has the reverse, or esterifying, action. (Treadwell.) It requires bile salts for its activity. It is present in human pancreatic juice as well as in pancreatin.

### Intestinal Juice

The secretion of the intestinal mucosa is at least partly under the control of the nervous system. Mechanical stimuli reflexly cause a flow of this fluid. It is probable that secretin exerts a hormonal effect for this secretion as well as for pancreatic secretion and for bile secretion. There is also a specific hormone, enterocrinin, secreted by the intestinal mucosa, which stimulates the mucosal glands. Both the volume of fluid and the content of enzymes are increased by enterocrinin. (Nasset, Pierce, and Murlin.) Intestinal juice is not as definite an entity as gastric or pancreatic juice, because it varies at different levels of the intestinal canal, and because its composition is not nearly as constant at different periods. Moreover, there seem to be two different types of secretion; one has digestive powers and the other, secreted periodically at about two-hour intervals, contains mucin and excretory products. It is usually quite turbid because of the presence of leucocytes, epithelial cells, and mucus. The total solids amount to about 1.5 per cent, about half of which is NaCl, NaHCO<sub>3</sub>, and other inorganic salts. It has a pH of about 8.3. The organic material comprises mucin and a number of different enzymes. Some of these enzymes are undoubtedly not actually secreted but are present in the leucocytes and epithelial cells, which disintegrate and liberate their enzymes. Moreover, since it is difficult to obtain intestinal juice, most studies have been on extracts of the mucosa. Hence we are not sure whether all the enzymes ascribed to intestinal juice are actually secreted or are in the mucosa, where they do their work as intracellular enzymes.

The saccharidases, maltase, sucrase (or invertase), and lactase, split the disaccharides, maltose, sucrose, and lactose, respectively, into their constituent monosaccharides. Enterokinase is the enzyme which transforms trypsinogen into trypsin. There is an amino-peptidase which splits off an amino acid from a peptide at the end having a free amino group.



There is also a dipeptidase. Thus, apparently, the final stage in the breakdown of the proteins to amino acids takes place. A lipase, which is activated by bile

Its, is present in the succus entericus of the dog and of man, but it is probably not of high activity. An amylase also occurs in the intestinal juice.

Three enzymes which decompose nucleic acid to its constituents are present here, also. They are the nucleases, phosphatases, and nucleosidases. The nucleases attack nucleic acids, releasing the mononucleotides in each (see Chapter 15). The phosphatases, which are not specific for nucleotides, hydrolyze them to phosphoric acid and purine or pyrimidine nucleosides. The nucleosidases complete the digestion of the nucleosides to purines, pyrimidines, and sugar. Some nucleosides are absorbed unchanged.

Thus intestinal digestion seems to be directed toward complementing the other digestive actions, finishing the hydrolysis of nucleic acid, disaccharides, simple peptides, and organic phosphates. The small molecules so produced are more readily absorbed.

### Bile

The bile is secreted, probably continuously, by the liver and passes into the hepatic ducts and into the common duct. It fills the gall bladder, via the cystic duct, and tends to distend all ducts and the gall bladder between digestive periods. The bladder wall, during these intervals, absorbs water from the bile contained in it, thus producing a highly concentrated bladder bile and making more space for the liver secretion. It also absorbs  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ , and perhaps other inorganic ions. Although bladder bile by this process is four or more times as concentrated as hepatic bile, both have the same osmotic pressure as that of blood serum. Gall bladder bile is slightly acidified in the process of concentration. The other constituents of bile do not appear to be absorbed to any appreciable extent by the normal gall bladder. The hepatic secretion is not under nervous control. It can be accelerated by various substances; secretin and bile salts are notable examples, bile salts being by far the more effective. These are usually referred to as "cholagogues." It has been suggested, however, that they be termed "choleretics," the word cholagogue being reserved for substances which stimulate the gall bladder to contract and thus bring about the flow of bile into the duodenum. The latter is under nervous, hormonal, and local control. Ordinarily the sphincter at the junction of the common duct and the duodenum is closed. The ducts are kept filled with bile by hepatic secretion, and upon increase of pressure the bile passes into the gall bladder. Although the gall bladder wall is very thin and has few muscle fibers, it probably does contract feebly and, perhaps, the sphincter of Oddi relaxes simultaneously. There seems to be a nervous mechanism for controlling this phenomenon, but it is not clear just how this acts. In regard to hormonal control, Ivy obtained an acid extract of intestinal mucosa which on intravenous injection caused contraction of the gall bladder. The hormone involved is not secretin but is apparently similar to it. It has been named "cholecystikinin." The two hormones can be separated by absolute alcohol extraction; secretin is thereby removed. Even more effective than the hormone as a stimulus for the discharge of bile is fatty food. Protein and carbohydrate have little effect, but emulsified fats like cream and egg yolk call forth a profuse discharge of bile. No explanation for this has been given.

**Composition of Bile.**—Human bile as secreted by the liver is clear, golden or brownish yellow in color, but sometimes olive green. It has a bitter taste and is a viscid slimy fluid. It is alkaline with a pH of from 7.8 to 8.6, but bladder bile may be even as acid as pH 6.5. The daily volume has been variously estimated at from 500 to 1,100 ml. In one case of biliary fistula there was an output of 525 ml. in twenty-four hours. Bile contains the following characteristic substances: bile pigments, bile salts, and cholesterol. There are, in addition, variable quantities of mucin, lecithin, inorganic salts, and urea. Hepatic bile contains about 1 to 4 per cent solid matter and bladder bile as much as 1 per cent. The high solid content of the latter is due to the absorption of water.

In Table XXV is given the range of various constituents in bile as found by several investigators.

TABLE XXV  
COMPOSITION OF BILE

	BLADDER BILE	LIVER BILE
Water	82.3-89.8	96.5-97.5
Solids	10.2-17.7	2.5-3.5
Bile salts	5.7-10.8	0.9-1.8
Mucus and pigments	1.5-3.0	0.4-0.5
Cholesterol and other lipids	0.5-4.7	0.2-0.4
Inorganic salts	0.6-1.1	0.7-0.8

**Bile Pigments.**—The bile pigments, bilirubin and biliverdin, give bile its color and are considered to be excretory products. Bilirubin predominates. Oxidation and reduction produce a series of varicolored compounds, some of which have received definite names.



The Gmelin test is based upon this color reaction. Concentrated nitric acid, overlaid with urine or other fluid which may contain bile pigments, will oxidize them at the junction of the two fluids, producing a rainbow of colors as a positive reaction.

The bile pigments are derived from the heme of the hemoglobin of “worn out” red blood cells. They are formed in the reticulo-endothelial cells. There are some cells of the reticulo-endothelial system in the liver, the von Kupfer cells, and consequently a small fraction of bilirubin originates in the liver itself. Most of the bile pigment, however, is produced by the reticulo-endothelial cells in other parts of the body, whence they are transported to the liver for excretion (Fig. 32). Involved in the formation of bile pigment are (1) denaturation of the globin of hemoglobin, (2) splitting off of the denatured globin, (3) the oxidation of the  $\alpha$ -methene carbon atom and the opening of the tetrapyrrole ring at this point, and (4) the removal of iron. (See page 239.) The iron removed is utilized for the manufacture of new heme. (Granick and Gildes, Drabkin.)

Biliverdin is next reduced to bilirubin by the addition of two hydrogen atoms at the double bond attached to the central  $\gamma$ -methene group. After bile passes into the duodenum, bilirubin is further reduced by the bacteria



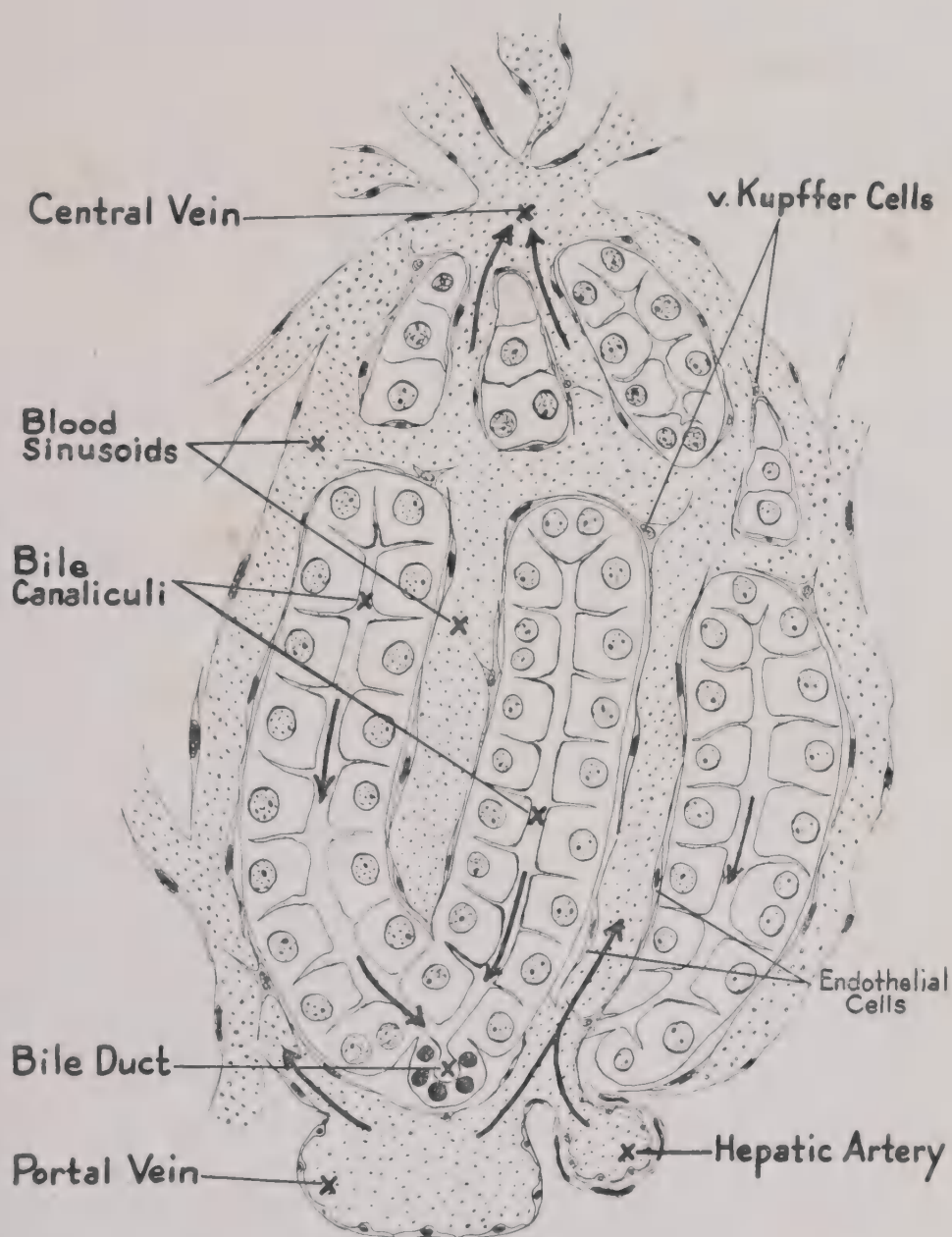


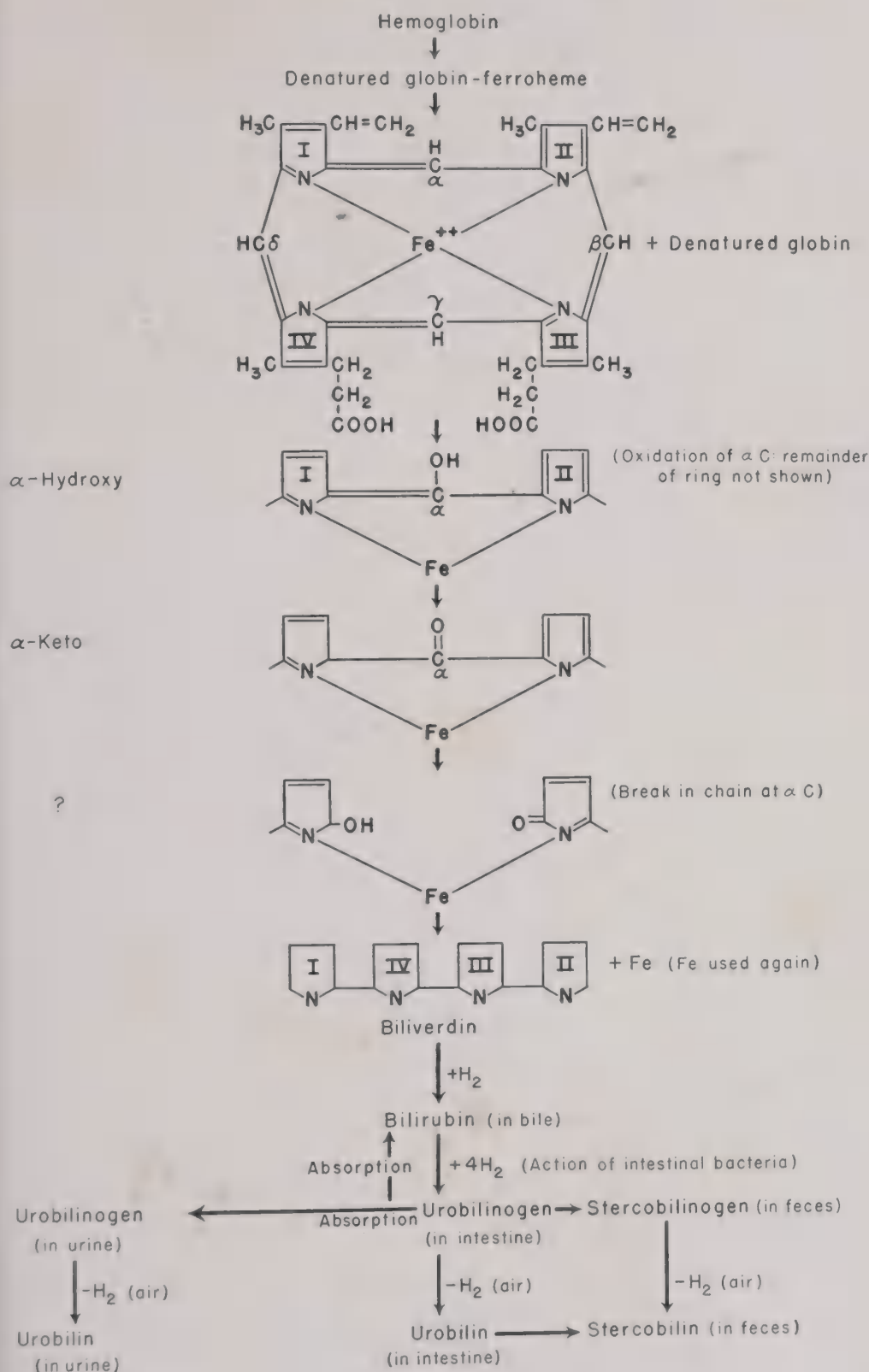
Fig. 32.—Circulation through liver lobule. Arrows show the direction of flow of the blood in the blood sinusoids toward the central vein. In the v. Kupffer cells bile pigment is formed to some extent and is secreted by the adjacent polygonal hepatic cells into the bile in the bile canaliculi. The direction of flow of the bile toward the bile duct is also indicated by arrows. (Courtesy Dr. Mary B. Stark.)

flora. One product is the colorless urobilinogen (mesobilinogen), in which four hydrogens are attached at the two double bonds of the chain and four more are used to convert the two vinyl groups to ethyl groups. Urobilinogen on contact with air, is oxidized to urobilin (mesobilin). Another reduction product is stereobilinogen, which is oxidized to stereobilin. The two latter compounds are excreted in the feces. Some authorities are of the opinion that they are identical with urobilin and urobilinogen, respectively. Some of the urobilinogen is absorbed by way of the portal circulation and is mostly eliminated again by way of the bile, after reconversion to bilirubin, but a small amount of it is excreted unchanged by the kidneys. In the urine the urobilinogen may be reoxidized, on standing, to urobilin. Schematically these reactions may be shown on the following page.

The theory that a bilirubin-globin complex circulates in the blood, and is normally split by "liver action," is being abandoned. This hypothesis was formulated to explain the excretion or nonexcretion of bile pigments in different types of jaundice, as well as to account for the direct and indirect van den Bergh tests (see page 597). There is, however, a specific bilirubin combining protein in blood plasma,  $\alpha_2$ -globulin. It is possible that this combines with bilirubin under certain pathological conditions and becomes a colloidal complex. Crystalloidal bilirubin, besides being easily secreted by the liver into bile, which is the normal process, is also easily excreted by the kidney if it is present in the blood. This occurs when the bile ducts are obstructed and the bile is dammed back into the liver and consequently into the circulation. Hence, in obstructive jaundice bilirubin is found in the urine. However, if for any reason there is an accumulation of colloidal bilirubin, this larger molecule will not be eliminated in the urine. Neither the normal liver nor the normal kidney can secrete this larger molecule. In hemolytic jaundice there is an overproduction of bilirubin, which is apparently largely converted to the colloidal form. Much of this circulates in the blood, but is not excreted by the kidney. There is also thrown into the intestine increased amounts of bilirubin. This is reduced to urobilinogen in the normal way, but in abnormally large amounts. Therefore, there is increased absorption of urobilinogen and excretion of it in the urine.

There are other types of jaundice in which there is primarily some damage to the liver. This damage may be caused by toxic agents, such as chloroform or arsphenamine, or by some acute or chronic liver disease. In such conditions there is not necessarily an increase in the production of bilirubin but the damaged or incapacitated hepatic cells cannot excrete it into the bile. Hence a considerable amount circulates and may be transformed into the colloidal form. No bilirubin appears in the urine but urobilinogen does because the urobilinogen, absorbed from the intestinal tract, cannot be re-eliminated via the bile by the poorly functioning liver.

**Bile Salts.**—The bile salts are chiefly the salts of glycocholic and taurocholic acids. These acids are combinations of cholic acid with glycine and taurine, respectively, joined together by means of peptide linkages. Cholic acid

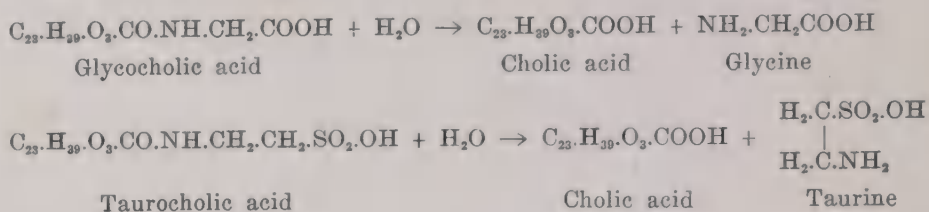


# Origin of Bile Pigments

Modified from Granick, S., and Gilder, H.: *Advances in Enzymology* 7: 305, 1947. New York, Interscience Publishers, Inc.)



is structurally related to cholesterol and probably is formed from it (see Chapter 17). The hydrolysis of each of these bile acids is as follows:



The structural relationship of taurine to both cysteine and methionine is very close. Experiments in which radioactive sulfur,  $\text{S}^{35}$ , was introduced in trace amounts into methionine showed that when this amino acid was fed to dogs, its sulfur was used in part to form taurine (Tarver and Schmidt). Cysteine, also, is converted into taurine. (See page 385.) Different species vary in the proportions and even in the nature of the bile salts found in their bile. (Haslwood and Wootton.) In man, besides cholic acid, linked to taurine and glycine, there are also present desoxycholic, lithocholic, and chenodesoxycholic acids.

**FUNCTIONS OF BILE SALTS.**—The bile salts are not excretory products. They are undoubtedly the most useful constituents of bile. After secretion into the intestinal tract they are absorbed almost completely and are carried by the portal blood back to the liver for re-secretion. Perhaps 10 per cent may be destroyed by bacterial action in the intestine or be lost by excretion. Several of their functions have been alluded to previously. They may be summed up as follows:

1. They accelerate the action of pancreatic lipase. This "nonspecific activation" transforms a relatively weak enzyme into a quite powerful one.
2. Because of their power of lowering surface tension, they aid in the emulsification of fats and tend to stabilize such emulsions. In fact, the bile salts, fatty acids, and lower glycerides are said to form one of the best emulsifying mediums for fats. This may permit the absorption of emulsified fat and leads to the presentation of a greater amount of surface to the lipolytic enzyme, and thus further aids its action.
3. The digestion of fats produces fatty acids and glycerol or lower glycerides. The fatty acids are not very soluble in the slightly alkaline medium present. However, the bile salts form complexes with the fatty acids which are water soluble and are easily absorbed. The entire complex may be absorbed and the bile salt again may be removed by the liver and re-secreted in the bile. However, it is also possible that some of the bile salt molecules remain at the surface of the mucosa and alternately combine with and release fatty acid molecules to the cell (Verzar and Kuthy). Thus a small amount of bile salts may do even more work than the part which must be taken to the liver and secreted in the bile.
4. They aid in the absorption of the fat-soluble vitamins. This is particularly important in the case of vitamin K.

5. It is the bile salts which keep cholesterol in solution.

6. They have a choleric action. Thus the liver is stimulated to secrete bile as long as bile salts are absorbed. This apparently continues during fat digestion and the absorption of the bile-salt-fatty acid complex; i.e., exactly during the period necessary for such secretion.

7. They stimulate intestinal motility.

**Cholesterol.**—The bile seems to be the chief channel for the excretion of excess cholesterol. The sources of biliary cholesterol are (1) synthesis by the liver, (2) decomposition of red blood cells, and (3) dietary cholesterol. Reabsorption of some of the cholesterol may occur, but not after it has been reduced. Reduction occurs by bacterial action, and the product is coprosterol, the sterol of the feces. Since cholesterol is not a very soluble substance, it is not surprising to find that it may precipitate out of solution from bile and form gallstones.

**Functions of Bile.**—To sum up the functions of bile, we may say that (1) it tends to neutralize the acid chyme, thus providing a more favorable hydrogen ion concentration for the enzymes secreted by the pancreas and the intestinal mucosa; (2) it aids in fat digestion in several ways; (3) it promotes the absorption of fat, the products of fat digestion, other lipids, and fat-soluble vitamins; (4) it has a choleric action; and (5) it is an excretory channel for bile pigments, cholesterol, certain drugs, metals, etc. Bile has no antiseptic properties; in fact, bacteria will grow in bile very rapidly. If bile is diverted to the exterior by a biliary fistula, the feces become clay colored, increased in amount, and greasy and have an extremely offensive odor. The color is, of course, due to the lack of stercobilin, and the greasiness and odor, to the undigested fat which has become rancid. Animals with such fistulas eventually develop abnormalities of the bones, associated with loss of inorganic salts, thus indicating some other function which is vital, since animals with bile fistulas do not survive very long.

The nontoxic emulsifying agents, some of the "new detergents" (page 81), have recently been tested therapeutically in cases of biliary and pancreatic deficiency. Here digestion and emulsification of fat are incomplete and the administration of these agents to cause emulsification artificially has been found to be helpful.

**Gallstones.**—Gallstones, or biliary calculi, are composed of material which has precipitated out of bile to form masses of varying size and shape. They usually are found in the gall bladder but may form in the bile ducts. If single, the stone is generally ovoid in shape, but if multiple, they have facets formed by pressing and rubbing against each other. When many are present, the shape of most of them is cuboidal. A gall bladder may contain as many as two thousand calculi. The color, hardness, and inner structure vary with their composition. When cut in cross-section a central nucleus, around which layers of the constituents are deposited, may be seen. Gallstones are usually classified, in regard to composition, into (1) cholesterol, (2) pigment, and (3) calcium carbonate stones. As a matter of fact, no gallstones are ever composed entirely of any one constituent. The so-called "pure cholesterol" stones may contain

from 90 to 98 per cent cholesterol, but there is always found some bile pigment and some inorganic salt. Stones in the human being belong almost always to the first two groups; that is, they are predominantly the cholesterol or pigment variety.

The mechanism of the formation of gallstones is not entirely clear. It was held for a long time, and is still rather generally accepted, that an infection or injury to the gall bladder mucosa produces a nucleus of microorganisms or a tiny clot around which cholesterol or pigment is deposited. It has been suggested that the proteins present in this nucleus carry an electric charge of an opposite sign to those present on cholesterol, pigments, and  $\text{CaCO}_3$ , and in that way bring about a precipitation. Since the gall bladder tends to concentrate bile, conditions are favorable for further precipitation. Bladder bile is normally more acid than liver bile. This would tend to keep  $\text{CaCO}_3$  in solution ordinarily.

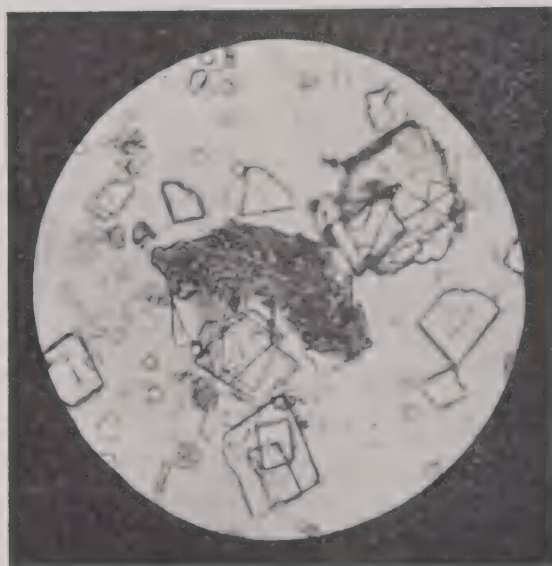


Fig. 33.—Sediment from "B" bile ( $\times 360$ ). The combination of cholesterol and calcium bilirubinate is indicative of gall stones. (Courtesy of Dr. Harry Barowsky.)

Infection, it has been shown, interferes with this acidification of bladder bile, leading to precipitation. A metabolic origin has been put forth as another explanation, the assertion being made that the high concentrations of blood cholesterol (hypercholesterolemia) are often associated with gallstones. It has also been suggested that the protective colloidal action of the mucin of the bile (together with the solvent action of the bile salts on cholesterol) may be responsible for the usual nonprecipitation of gallstone constituents, and any factor which disturbs the balance is likely to initiate the formation of minute concretion. Once started, as stated before, the number and size increase more or less rapidly. It has been shown by Rains and Crawford that the bile salts, in solutions of increasing concentration, show definite alterations in surface tension and conductivity, which denote a change of phase. This is taken to indicate ionic aggregation or micelle formation. Such con-



centration can, and does, occur in the gall bladder. Consequently, the cholesterol dissolving power of the bile would be decreased, and precipitation of cholesterol would be explained.

**Duodeno-Biliary Drainage.**—This technique is used to attempt to obtain information regarding the duodenum, pancreas, liver, gall bladder, and the various ducts carrying their secretions to the intestine. A small caliber rubber tube is passed, first, into the stomach, at which stage the gastric residuum may be obtained and examined. Then the tube is permitted to go through the pylorus to the duodenum. Duodenal contents and then bile may be obtained when the tip of the tube has reached the sphincter of Oddi. The operator may assure himself of this position by use of a fluoroscope. According to the method of Lyon, the fasting duodenal contents are first removed and then a measured volume of saturated magnesium sulfate is introduced by means of a glass syringe. Part or all of it is removed by suction, and then the fluid flows by syphonage. When the bile begins to appear it is usually seen to be light, golden yellow in color. This is designated "A" bile and is considered to be the bile present in the common duct. It usually amounts to from 10 to 30 ml. When the bile begins to show a darker color, it is collected separately as "B" bile. This is presumed to be gall bladder bile. When this changes again to a lighter shade, usually even lighter than the "A" bile, it is collected as "C" bile and is probably bile just as it is secreted from the liver. "A" bile is usually golden yellow, "B" is darker yellow to brown, and "C" is lemon yellow. Abnormally they may be quite different. Olive oil is often used instead of magnesium sulfate as a relaxing agent for the sphincter of Oddi. It has the advantage of permitting a more exact determination of volume. However, it has the disadvantage of changing the surface tension, which one may wish to determine as a means of estimating the amount of bile salts present. Obviously, even under the best conditions, one cannot be sure that the fluid obtained is pure bile. Duodenal fluid, gastric juice, and pancreatic juice may be mixed with it. However, even under such circumstances, certain information, such as the absence of bile salts or the absence of "B" bile, may be of decided importance. A microscopic examination should be made and may prove to be of great value. This is particularly true of "B" bile. The appearance of pus cells, bacteria, and characteristic crystals aids the clinician in diagnosis and treatment. Fig. 33 is a typical photomicrograph of abnormal sediment from "B" bile.

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## Chapter 11

# CHEMICAL CHANGES WITHIN THE LARGE INTESTINE

The processes which go on in the large intestine are due mostly to the activity of the myriads of microorganisms which live and die there. These enter the tract with food and saliva and may survive passage through the stomach since the HCl is not always present in bactericidal concentration. Consequently some living microorganisms pass into the small intestine and they begin to multiply as the reaction becomes more favorable. However, even near the ileocecal valve the intestinal contents do not contain large numbers of such organisms. At this point there are present some undigested food residues, unabsorbed secretions, such as bile and pancreatic juice, and cell detritus. Analysis of this material shows it to have about the same amounts of nitrogen, fat, and carbohydrate (based on dry weight) as normal feces, but it is not fecal-like. The pH is about 5.9 to 6.5.

In the large intestine these materials are transformed into feces. A number of enzymes are possibly present in the secretion of the mucosa of the large gut, but digestion by them is generally believed to be of little importance. This secretion is alkaline and viscid and undoubtedly tends to bring the contents over to the alkaline side. The conditions for bacterial growth (particularly anaerobic) are excellent: there are warmth, darkness, little oxygen, an almost neutral medium, and food material in a semisolid condition. The organisms flourish, utilize the food materials, transform them into their own protoplasm, multiply, and die. In fecal material, from one-fourth to one-half of the dry matter is said to be made up of living and dead bacteria. Water is absorbed by the mucosa and the characteristic consistency results.

In the newborn infant the first fecal discharge is termed "meconium." This is a very dark brownish-green semisolid material. It consists of intestinal and biliary secretions which have accumulated in the large intestine from the fourth fetal month on. Meconium continues to be passed for the first three or four days after birth and accounts for much of the loss of weight which occurs at that period. Usually with the ingestion of milk there is seen a gradual change to the usual type of infant feces. These are soft, golden yellow to greenish-yellow in color and have an acid reaction. The odor is slightly "sour" but not



unpleasant. The approximate general composition of stools of the infant and of the adult is as follows:

	STOOL OF	
	BREAST-FED INFANT	STOOL OF ADULT
Water (per cent)	85	75
Organic solids (per cent)	13	20
Ash (per cent)	1	5

In the feces of infants there is found very little of the milk protein but rather large amounts of fat, fatty acids, and soaps.

### General Character of Feces of Adult

Adult fecal material is normally brown, varying in color with fat and water, which lighten the color, and bile pigments, which darken it. About 80 to 170 grams of feces are eliminated per day. The composition varies greatly and not much significance can be attached to analytical findings. This is easily understood when it is realized that feces contain undigested, indigestible, and unabsorbed food residues, secretions of the gastrointestinal tract, bile constituents, and desquamated epithelial cells. Included in the unabsorbed food may be rather large amounts of iron compounds, since the intestine will not absorb more than a certain amount of iron administered. There is also a variable amount of phosphate, depending upon the precipitation of this ion in insoluble form. A large amount of calcium is found in feces either as the phosphate or the oxalate. In fact, more calcium is present in feces than in urine. The secretions contain lipids which have been shown to be of the same types as the blood lipids and are now recognized as having been actually secreted by the mucosa of the small intestine.

The bile derivatives in feces are stercobilin, a transformed bile pigment which gives the stool its brown color, and coprosterol. Coprosterol is a reduced sterol, coming largely from the cholesterol of bile.

The pH of the stools of healthy adults on a mixed diet varies from 7.0 to 7.5 according to Robinson. Common laxatives, even magnesium oxide, tend to change this to the acid side, but it is questionable whether the contents of the large intestine can be changed to an acid reaction by feeding acid-producing bacteria. This was the rationale of feeding fermented milk products, such as acidophyllus milk, etc. It was held that by implanting such organisms in the large intestine an acid flora could be established which would have a favorable effect upon health.

**Intestinal Gases.**—The volume of gas present in the gastrointestinal tract of the human being is variable but averages 1 liter. Since some of this is absorbed, the total volume expelled in the course of a day is somewhat less than a liter, but may be as much as 2,600 ml. or as little as 12 ml. (Blair, Dern, and Bates.) The components of the mixed gases will vary with the diet. On a high milk diet the predominant gas is hydrogen; on a vegetable

diet, methane; and on a meat or mixed diet, nitrogen. In all cases, all of these gases, as well as  $\text{CO}_2$  and usually  $\text{H}_2\text{S}$ , are present. The nitrogen is derived from swallowed air, from air dissolved in food and drink, and from nitrogen which diffuses out from the blood in the blood vessels of the gut. It is in solution in the blood, having passed into it during respiration. A typical analysis of the gases obtained from a normal man was:  $\text{N}_2$ , 59.4 per cent by volume;  $\text{CH}_4$ , 29.6 per cent;  $\text{CO}_2$ , 10.3 per cent; and  $\text{O}_2$ , 0.7 per cent. (Fries.) In intestinal obstructions there accumulate gases of somewhat similar composition, except that there seems to be less methane and traces of hydrogen and hydrogen sulfide. (Wangensteen.) Probably the amount of  $\text{H}_2\text{S}$  will depend upon the presence of the sulfur-containing amino acids and the type of decomposition which they undergo. The concentrations of  $\text{CO}_2$  and  $\text{H}_2\text{S}$  are low because these gases are quite soluble in the aqueous medium and therefore are absorbed rather easily.

Intestinal gas may cause considerable distress because of distention of the abdominal viscera. This is increased when atmospheric pressure is diminished, as at high altitudes, and serious symptoms may result. (Ivanov.)

### **Action of Microorganisms on Carbohydrates and Fats**

Bacteria, yeasts, and other organisms in the large intestine probably act upon carbohydrates present producing butyric, lactic, and perhaps other organic acids, ethyl alcohol,  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{H}_2$ . How much absorption of any of these takes place in the human being is not known. It is fairly certain that cellulose is not transformed to glucose and the latter absorbed and utilized as was formerly believed to be the case.

The action of intestinal organisms on fats, if any, is probably simple hydrolysis. Little is known about this, however, and the same is true of the action of the organisms upon fatty acids and glycerol.

### **Fecal Lipids**

Normally the lipids of the feces have little, if any, direct relation to the lipids of the diet. As stated above they resemble the lipids of blood and are undoubtedly secreted by the intestinal mucosa, chiefly that of the small intestine. Abnormally, marked increases in the lipids of the feces may occur. In such instances the lipids are food lipids, not those secreted by the liver or intestinal mucosa. These increases may be due to blockage of the bile ducts, the pancreatic ducts, or both; to failure of the pancreas to secrete pancreatic juice, or to imperfect absorption (for example, when there is increased motility of the upper intestine and the food rushes through too rapidly). Conditions in which the feces contain large amounts of fat, fatty acids, and soaps are called "steatorrheas."

The normal values for fat and its derivatives in feces will vary widely. The range is from 7 to 25 per cent for "total fat"; that is, neutral fat, free fatty acids, and soap. A figure of over 25 per cent is considered abnormal and requires more detailed study. If the neutral fat is high, one should suspect that there is deficient fat digestion, and if the total split fat (i.e., the sum of soaps and free fatty acids) is above its usual percentage, there is probably some abnormality in the absorptive process. In this way a fractional fecal analysis may aid in diagnosing obscure gastrointestinal conditions. In steatorrhea there are frequently found lesions of the bones similar to rickets, sometimes dwarfism, etc. A low serum calcium is usually present and sometimes tetany results. This seems to result from an excessive loss of calcium in the feces along with the fat. This must be due to a lowered absorption of calcium from the intestinal tract which may be accounted for in one of three ways: (1) The excess of fat in the tract holds the fat-soluble vitamin D there and prevents its absorption; vitamin D is concerned in the absorption of calcium from the gastrointestinal tract. (2) The intestinal wall may be impermeable to calcium ions in these conditions. (3) The fatty acids form insoluble soaps with the calcium and these are excreted in the feces as such. There is some evidence for each of these three possibilities.

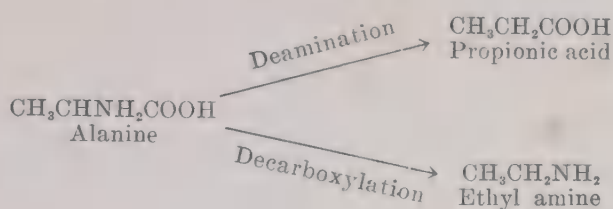
In addition to neutral fats, fatty acids, and soaps, there are always found various sterols in feces. These include cholesterol and its reduction product, dihydrocholesterol and coprosterol. They are true secretions eliminated by way of the bile or through the intestinal wall, but in addition there are also unabsorbable plant sterols as well as any excess cholesterol of the diet which has escaped absorption.

### Action of Microorganisms on Proteins

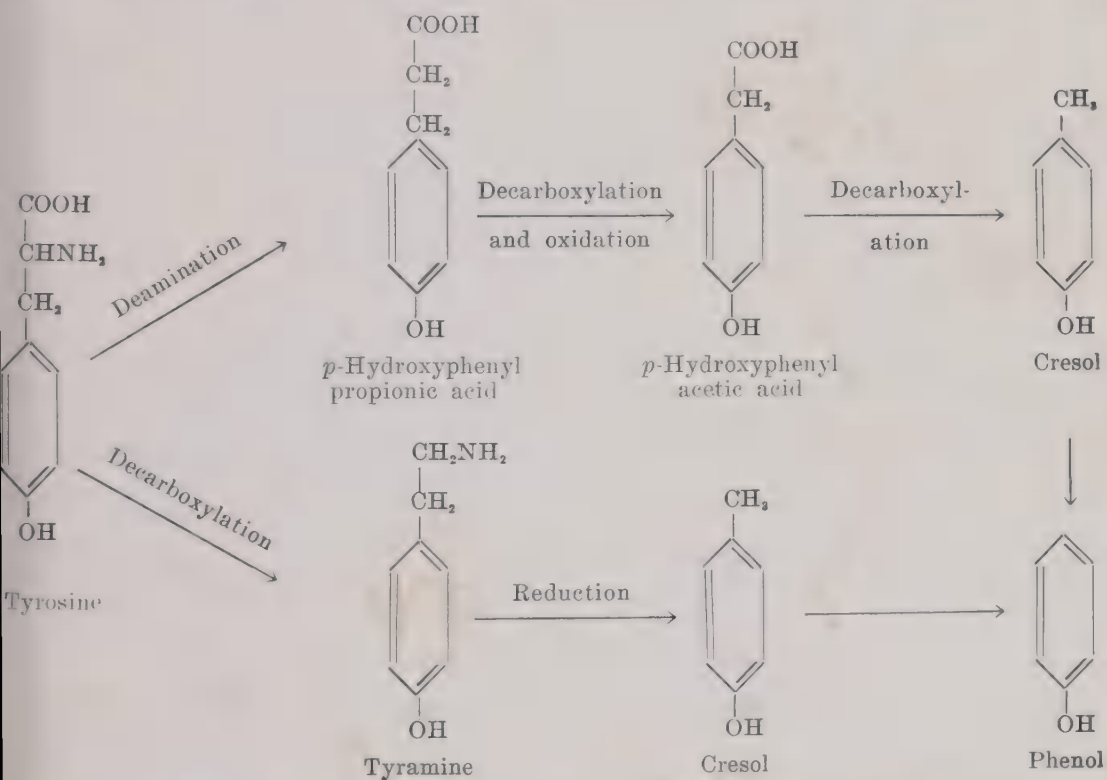
**Putrefaction.**—The decomposition of proteins by anaerobic organisms is termed putrefaction. The proteins which reach the large intestine may be undigested or partly digested food residues, unabsorbed amino acids, or cellular detritus. In addition, there are the proteins of the dead bacteria. The action of microorganisms upon this varied assortment begins with a digestive action. There may be proteases, such as trypsin, which have not been destroyed, proteases from disintegrated epithelial or bacterial cells, or the active enzymes of the living bacteria. Proteolysis results, of course, in the formation of free amino acids and since little or no absorption takes place in the large intestine, they are attacked by the microorganisms to a varying degree and in two general ways. These are decarboxylation and deamination. Oxidations, reductions, and hydrolyses also occur. These reactions are all the results of the appropriate enzymes. If deamination occurs first, acids are formed, while amines result from



decarboxylation. The simpler amino acids yield simple organic acids or amines, as the case may be. Thus, alanine forms propionic acid or ethyl amine.

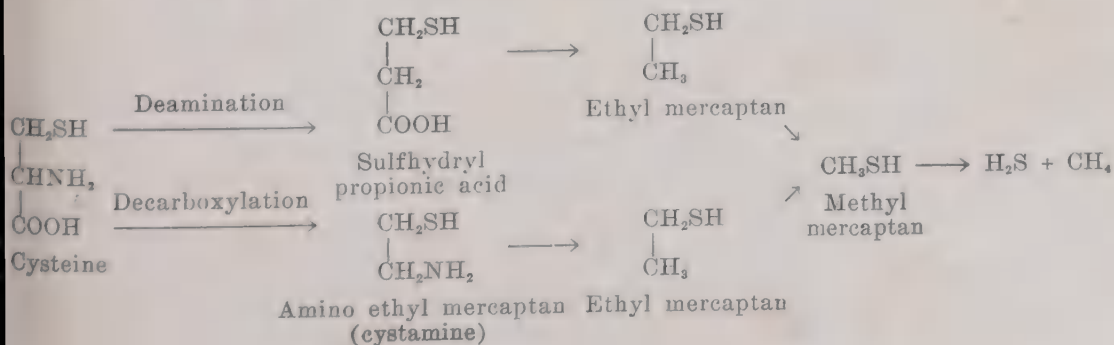


The amino acid tyrosine may undergo decomposition along two routes also.



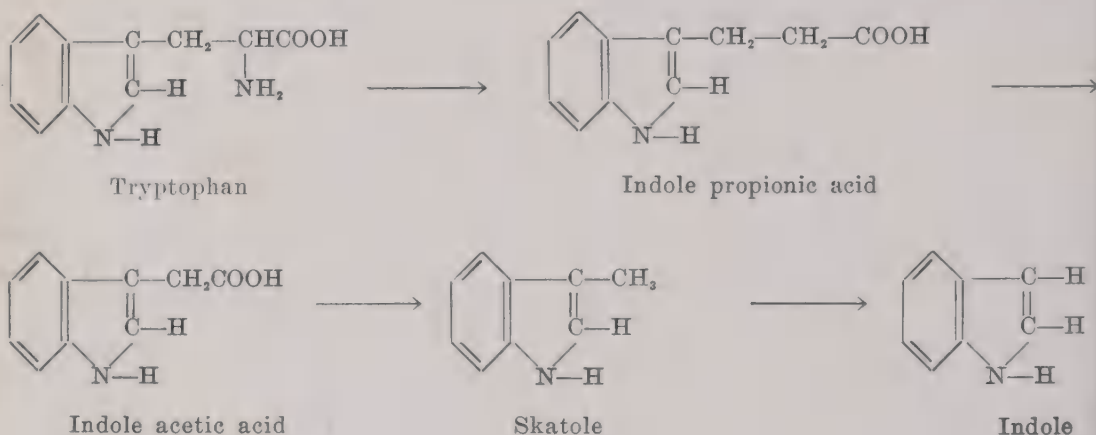
It will be noted that phenol is the ultimate product of both series of reactions, but the first series converts the amino acid to an acidic intermediary, while the second is through a basic one, an amine.

The two types of action on cysteine are:

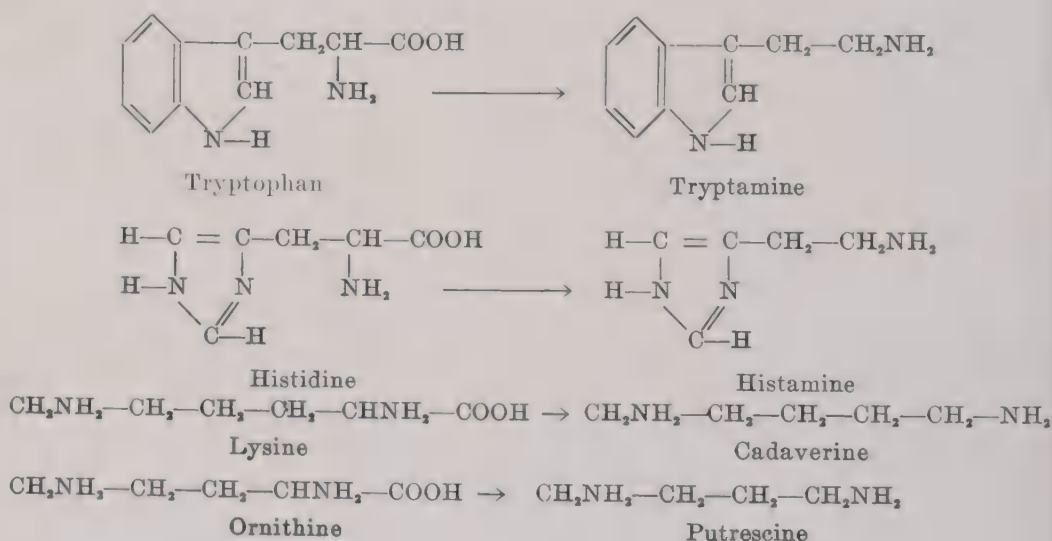


This indicates how, by (1) first deaminating and then decarboxylating and (2) first decarboxylating and then deaminating, different intermediate products are formed. These, if further attacked by the bacterial enzymes, yield finally the same products.

The products of tryptophan deamination are quite important.



Indole and skatole are the two substances which give the characteristic foul odor to feces. If first decarboxylated, tryptophan yields tryptamine. This and other amines which may be formed in the large bowel are:



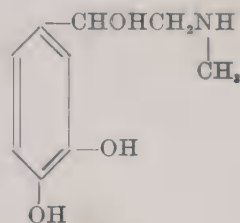
### The Question of Autointoxication

Most of the amines, when introduced into the blood stream in appropriate dosage, have marked pharmacological effects. Histamine, as has been seen, stimulates the gastric glands, and, especially in large doses, it also has a blood pressure-lowering effect. This depressant action is shared by several other amines produced in the large intestine, namely, putrescine and cadaverine. On the other hand, tyramine raises blood pressure. This is particularly interesting

in view of its structural relationship to adrenaline, the hormone of the adrenal medulla which has a pronounced blood-pressure-raising action. Tryptamine also raises blood pressure after a preliminary depressor action (Page).

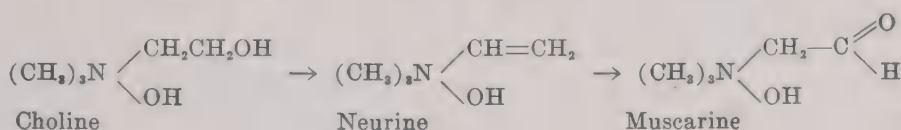


Tyramine



Adrenaline

From lecithin and sphingomyelin, choline is split off, and this is converted to neurine and muscarine by anaerobic organisms.



Choline is slightly toxic if administered to animals; neurine and muscarine rather more so.

From cephalin, serine is derived, and this on decarboxylation yields aminoethyl alcohol, colamine.



Colamine is only slightly toxic but amino ethyl mercaptan has a marked blood pressure-lowering effect and this is formed during the bacterial decomposition of cysteine.

It is probable that diarrhea may result from an overabundance of some of these products of intestinal putrefaction, particularly the acidic compounds. The question arises, however, as to whether the other toxic products, the amines, the choline derivatives, the mercaptans, etc., are harmful. The idea that the absorption of some of these products of bacterial activity is the cause of many of the ills of mankind has long been prevalent among medical men and, particularly, among laymen. There is no question that some of these products are somewhat toxic when administered orally and even more so if given parenterally; that is, by any route other than by mouth. It is doubtful, however, if large enough amounts are ever absorbed to produce harmful effects. For example, the total amount of indole in the feces is seldom over 60 to 70 mg., and yet 1 gram given by mouth produces no ill effects and 2 grams cause only a slight headache and dizziness. Small amounts of indole and skatole are often absorbed, as evidenced by the excretion of their detoxication product, indican, in the urine. They are therefore fully detoxicated. Sherwin and



Power have reported that the amines produced in putrefaction may be introduced into the gastrointestinal tract in amounts much greater than occur in constipation without the appearance of unusual symptoms. Regarding other toxic products formed by bacteria in the large intestine, they are (1) not absorbed in appreciable quantities, (2) destroyed by the mucosa of the intestine or (3) detoxicated in the liver or some other organ.

What is the basis then for autointoxication, the symptoms of which often accompany constipation—mental laziness, malaise, headache, dullness, coated tongue, poor appetite, and “biliousness”? Evidently the old idea that they are caused by absorption of toxic materials from the sluggish intestine is untenable. Alvarez is of the opinion that most of these symptoms result from mechanical distention and irritation of the rectum by the fecal masses and their effects are caused by reflex action. Many of the symptoms can be reproduced by simply packing the rectum with cotton. Probably small waves of contraction originate at such packed locations and travel in a reverse direction up the intestinal tract (antiperistalsis) and thus give rise to foul breath, coated tongue and other symptoms. It is not to be assumed that absorption of toxic products from the intestine never occurs. Probably it does, but the symptoms usually associated with constipation and with what has been termed autointoxication in the past are not caused by products absorbed from the large intestine. Absorption of toxins is more likely to occur in diarrheal conditions, but even in these cases the toxic action of these substances is of little moment.

**Ptomaines.**—A number of these toxic products have been classed together as “ptomaines.” These include muscarine, neurine, cadaverine, and putrescine. If food, particularly meat or fish, decomposes under the influence of putrefactive organisms, these compounds are formed. Usually this results from inadequate refrigeration of food or its storage for too long a time. If such spoiled food is eaten, they are likely to cause “food poisoning.” Possibly this results because these toxic products are absorbed more readily from the small intestine than from the large. However, the body can detoxicate fairly large quantities of them. Usually food poisoning means an infection carried by infected food into the gastrointestinal tract, and the pathological effects can be explained by assuming the *continued formation* and absorption of the toxic products plus specific toxins elaborated by the bacteria.

**Pathological Constituents of Feces.**—In order to detect abnormal constituents, the stools may be examined macroscopically, microscopically, and chemically. Each of these methods may yield valuable information. For example, simple observation, after mixing with water, and straining through cheesecloth, may enable one to find gallstones, undigested food residues, mucus, epithelial shreds, and, rarely, intestinal concretions. Intestinal concretions are chiefly inorganic, usually  $\text{NH}_4\text{MgPO}_4$ , with some admixture of  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{CaCO}_3$ ,  $\text{CaSO}_4$ , protein, or calcium or magnesium soaps. They always have a nucleus of some indigestible substance, such as hair, or even gallstone.

Microscopically, one may see crystals which might indicate the presence of salts or organic compounds which are ordinarily absorbed. Undigested

food, such as fat globules, meat and vegetable fibers, starch granules, etc., are often observed.

Chemically the quantitative estimation of fat, fatty acid, and soaps is sometimes required, but a strict control of the intake is then important. A qualitative test for unchanged bile pigments is rarely positive, except in severe diarrhea, when the intestinal contents are rushed through the tract. Ordinarily they are converted into stercobilin and stercobilinogen. The most important chemical determination is the qualitative one for blood. Among other things, this aids in the diagnosis of gastrointestinal ulcers and malignancies. A chemical test is often necessary because the colors which blood imparts to feces vary from bright red to black. A small amount of reddish-black in the brown feces is indistinguishable by the naked eye. The color of blood in the feces depends upon the length of time the blood remains in the small intestine—not on the site of the hemorrhage. Blood from the duodenum will stain feces red if it moves through the small intestine fast enough, because the darkening mechanism requires time and takes place solely in the small intestine. (Hilsman.)

It is therefore often necessary to test for "occult" blood; i.e., blood which is not macroscopically evident. This may be done most simply by suspending a small amount of feces in 5 ml. of water, boiling to inactivate the oxidizing enzymes, and then applying to the cooled fecal suspension any of the standard tests for blood; e.g., the benzidine, reduced phenolphthalein, orthotolidine, etc. If a positive reaction is obtained with a patient on a mixed diet, the test should be repeated after he has been on a meat-free diet for a sufficient length of time.

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## Chapter 12

# VITAMINS

### Historical

At about the turn of the century students of nutrition considered that a well-balanced diet need contain only a suitable amount of each of the following "proximate principles": proteins, carbohydrates, fats, inorganic salts, and water. Many investigations based on this premise were undertaken to determine the quality or amounts of these several ingredients or to vary their proportions. To accomplish this, animals were fed on highly purified foodstuffs and invariably the experiments resulted in failure. Perhaps the earliest of these was the work of Lunin, in Bunge's laboratory at Basle in 1884. He fed young mice on the purified proteins, sugar, fats, and salts of milk and found that they did not grow. He expressed the opinion that some "unknown substances" must be present in milk without which normal health and growth could not be maintained. This work did not attract much attention and was soon forgotten. In 1897, Eijkman, a Dutch physician working in Java, came to the conclusion that the oriental disease beriberi resulted from an imperfect diet which consisted chiefly of polished rice. This was not only a clinical observation, but was also experimentally proved on fowls. When chickens or pigeons were fed for some time on polished rice they developed a polyneuritis. Both patients and birds could be cured by adding the polishings of the rice to the diet. Eijkman explained this by assuming that white rice contained a toxin which could be neutralized by an antitoxin present in the husk of the rice. When Eijkman returned to Holland, the investigation was taken up by Grijns in 1901. He repeated and enlarged on Eijkman's work. He was not able to extract a toxin from polished rice; he showed that there was actually a protective and curative substance in the polishings, and also in other foods. Grijns was probably the first to have a clear conception of a deficiency disease and to attempt to isolate an active principle from foods.

These experiments led Pekelharing to again perform experiments with purified foodstuffs. He fed them to mice and found that, although at first they ate well and seemed healthy, after about four weeks all died. However, if milk, or even whey, was given instead of water, they thrived upon the diet. He concluded that "an unrecognized substance occurs in milk which is of paramount importance for nutrition, even in minute quantities." This was published in a Dutch journal in 1905 and did not become widely known. The same type experiment was performed independently by Osborne and Mendel in this country and the same conclusion was reached. They were studying the nutritive value of highly purified proteins isolated from various cereals. They also could obtain no growth, or even maintenance of weight, in young rats unless "protein-fr



milk" was added to the diet. These experiments were done in 1911, and McCollum in this country and Hopkins in England made similar observations at about the same time. It was also in 1911 that Casimir Funk isolated from rice polishings a crystalline substance which was efficacious in preventing or curing polyneuritis in pigeons. His analyses indicated that it contained nitrogen in a basic form and that it was probably an amine. Since it appeared to be essential to life, he named it "vitamine." The spelling has since been changed to "vitamin" and has been applied to a whole series of substances found in foods, without regard to their chemical structure.

More of this absorbing chapter of biochemical history cannot be detailed here, nor can the names of the other brilliant investigators in this field even be listed. The interested reader may consult the references at the end of this chapter.

The experimental animal hitherto most widely used in vitamin work is the white rat. This animal has been bred and studied at the Wistar Institute, and its normal average growth curves and "vital statistics" have been described. Moreover, it responds in very characteristic fashion to many food deficiencies. Other animals have been used, partly because the rat need not ingest all nutritional factors and partly because substantiating or supplementary evidence is required. Thus the guinea pig is used in vitamin C studies because the rat does not require much, if any, of this factor in its food. Pigeons are the classical test animal for demonstrating the polyneuritis caused by lack of vitamin B<sub>1</sub>, and dogs, mice, ducks, chickens, hamsters, roaches, and particularly microorganisms are used for other vitamin studies. In fact the necessity today is to use species other than the rat in order to discover new factors for which the rat may not be a good test animal. One method of demonstrating a vitamin deficiency is to feed a group of animals a diet adequate in all nutritive factors except the single substance in question. A control group must be fed the same diet plus a sufficient amount of the substance omitted from the diet of the experimental group. All animals are weighed at regular intervals and their physical condition is carefully observed. After a time, the animals on the deficient diet may stop gaining weight and later they may lose weight; if the vitamin needed is not restored to their diet in time, they may die of malnutrition. Sooner or later, after they start to lose weight, they begin to develop symptoms characteristic of the particular avitaminosis (vitamin deficiency) concerned. Often these symptoms may be alleviated by administering the required vitamin, but in prolonged avitaminoses the pathological lesion has become so fixed that it is irreversible. This general method, the biological method, or "bioassay," is the one originally used to determine whether a given food contained a given vitamin, and it can be made fairly precise. The biological method usually requires weeks before an answer is obtained. When the chemical structure and chemical and physical properties of an individual vitamin are known, quantitative chemical methods are used, but some claim that biological assay is the only correct way to determine the vitamin content of any food because a vitamin may be present and react chemically but still be unavailable nutritionally. The chemical methods depend, of course, upon some

outstanding chemical property of the vitamin, such as the production of a color, lending itself to colorimetric estimation, or the reducing activity, which can be rather accurately measured. Nowadays microbiological methods are being used whenever possible because of their rapidity and economy. The principle of these methods is the same as that of the microbiological methods for the determination of amino acids, described on page 123. In this case all the amino acids and all other nutritional factors, except the vitamin studied, are included in the medium, and the amount of growth will depend upon the amount of vitamin in question in the added "unknown." It is understood that the particular organism used must be susceptible to the lack of the nutritional factor being assayed.

Intestinal organisms play a role in the vitamin quota available to the animal or man. They may synthesize vitamins in significant amounts. Vitamin K is a very important example, but folic acid, nicotinic acid, pyridoxine, biotin, thiamine, and riboflavin are others. These may be absorbed to a varying extent and utilized. This fact renders rather inaccurate the figures for "daily requirements" of the different vitamins in the following pages, but it is recommended that one should ensure an adequate intake of each vitamin and rely very little on intestinal synthesis. Certain organisms have the opposite effect; that is, they destroy vitamins. Adding certain antibiotics and sulfa drugs to the feed of domestic animals benefits growth; this increased growth may be due to a selective bacteriostatic action upon the organisms which destroy vitamins. (See page 304.)

**Definition.**—A vitamin is a naturally occurring essential organic constituent of the diet which, in minute amounts, aids in maintaining the normal activities of the tissues. The vitamins differ from the hormones in that the former are supplied to the body chiefly from the food eaten, while the latter are manufactured by the body's own glands. Since both act when present in extremely small amounts, the question as to how they differ from enzymes may very well be asked. The answer is that quite probably most vitamins and hormones enter into enzymic systems in order to effect their physiological functions. Some are known to be coenzymes, and their structure and action are being studied. However, it is not necessarily true that all enzymes or coenzymes inherently contain a vitamin or a hormone to complete their chemical structure.

Very early in the study of vitamins they were classified as to their solubility. It was at first thought that there was only one "vitamine" and later that there were two; a fat-soluble A and a water-soluble B. Then B and C were differentiated as water-soluble entities, components of the earlier "B," and A was found to be a mixture of two fat-soluble substances, which were named A and D. As their curative powers came to be recognized, they were given the subtitles indicative of their action in this respect: vitamin A, fat-soluble A, the antiophthalmic vitamin; vitamin C, water-soluble C, the antiscorbutic vitamin; and so on. Some of the materials proved to be mixtures, and individual factors having different effects from the main action of the original extract were isolated. An example is the B vitamin group. In other instances a series of compounds, close

related chemically, proved to have similar effects, so that, for example, one should not speak of vitamin D but of the vitamins D, since there are several of them. The structures of all the commonly known vitamins have been worked out and some of them are manufactured on a large scale, either by chemical or fermentation processes. Others are still obtainable only from natural sources.

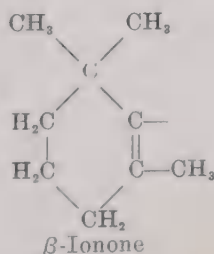
## THE FAT-SOLUBLE VITAMINS

The fat-soluble vitamins include vitamins A, D, E, and K.

### Vitamin A

**Properties.**—Vitamin A is soluble in fats and in fat solvents. It is stable to rather high temperatures, except when the conditions are favorable for oxidation, and in ordinary cooking or canning operations it is harmed but little. It is destroyed by exposure to ultraviolet light. It is available to the human organism either in the form of the vitamin itself or of a precursor, one of a series of carotenoid pigments, commonly called carotenes. The carotenes form part of the pigments of many green and yellow vegetables. After absorption they are converted into the vitamin in the intestinal wall. It is therefore not surprising that the sources of the vitamin itself are of animal nature. The carotinoids are not all equally potent in their ability to form vitamin A. Beta-carotene is most effective since, as will be seen from the formula, it is a symmetrical compound, each half of which is convertible into a molecule of the vitamin. Other members of this group which lead to the formation of vitamin A, but only half as much as beta-carotene, are alpha-carotene, gamma-carotene, and cryptoxanthine. Vitamin A has a characteristic absorption spectrum, with a band at 325 to 328  $m\mu$ —quite different from that of carotene, which has no absorption band in that region. It also gives a beautiful color reaction, an intense blue, when treated with sodium trichloride. Carotene under the same conditions yields a greenish-blue color. This reaction has been used for quantitative determinations of the vitamin. Recently vitamin A in aqueous dispersions has been recommended for clinical use since it is claimed to be more rapidly absorbed than its oil solutions.

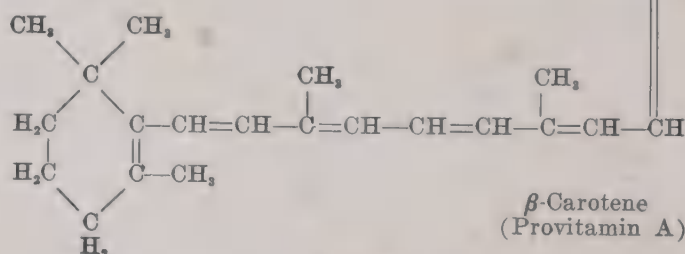
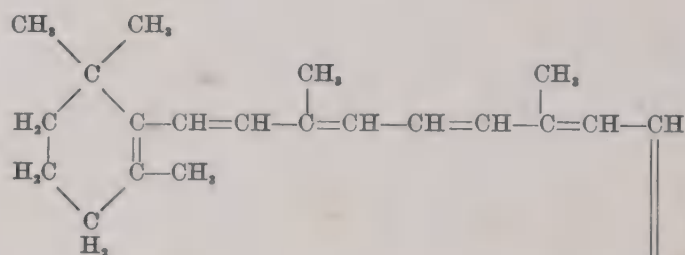
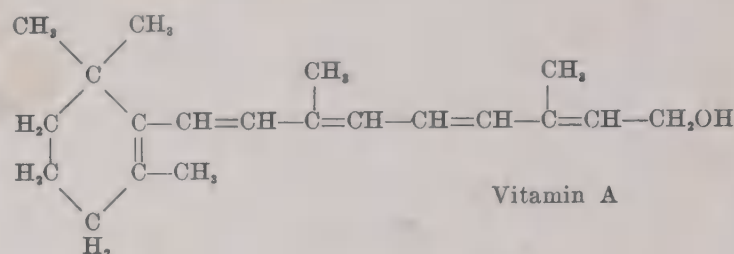
**Structure.**—Vitamin A is a complex primary alcohol, with the empirical formula  $C_{20}H_{29}OH$ . It was isolated in 1931 by Karrer and his associates and was synthesized by Milas in 1946. It contains a  $\beta$ -ionone ring:



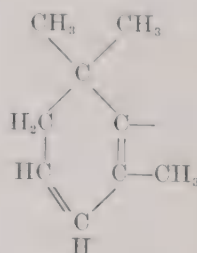
Beta-carotene has two such rings and no alcohol groups. The other pigments mentioned each have one  $\beta$ -ionone ring and one group very similar to it. It is thus apparent why they can yield only half as much vitamin A as  $\beta$ -carotene.



does, since each molecule of  $\beta$ -carotene may give rise to two vitamin A molecules. The structures of  $\beta$ -carotene and vitamin A, as worked out by Karr and associates, are:



There are actually two vitamins A, known as vitamin  $A_1$  and vitamin  $A_2$ . The formula shown is that of  $A_1$ . Vitamin  $A_1$  predominates in the livers of the cod and other salt-water fish and  $A_2$  in those of fresh-water fish. The physiological activity of both seems to be the same qualitatively, and it is justifiable to call them both vitamin A, although  $A_2$  is much less active in promoting the growth of rats than is  $A_1$ . Vitamin  $A_2$  has the following ring structure instead of that for  $A_1$  (Farrar).



**Occurrence.**—The best sources of the vitamin are cod-liver oil and other fish liver oils, fish roe, the flesh of oily fish, the livers of other animals, butter, eggs, and cheese. The provitamin occurs most abundantly in carrots and other yellow vegetables such as squash, sweet potatoes, and many green vegetables, particularly broccoli, spinach, and beet greens. In fish liver oils vitamin A is present as esters of fatty acids, chiefly stearic, palmitic, and higher unsaturated

acids. When the liver oil is ingested by man or animal, the vitamin A esters are hydrolyzed in the intestinal wall. (In those conditions in which the individual is unable to hydrolyze esters, such as celiac disease and tropical sprue, severe deficiencies of vitamin A may occur.) The vitamin is recombined with fatty acids *characteristic of the host* immediately after passage through the gut wall. Exactly which esterases perform these functions is not known, but apparently it is essential for the esters to be split and they cannot be absorbed, unhydrolyzed, in the way that a part of the fats apparently are. (Gray.) The vitamin esters are then conveyed by the portal vein to the liver where they are stored in ester form. From the liver, vitamin A is redistributed to the various organs, by way of the blood stream, partly as ester and partly as free alcohol.

After the provitamin is absorbed, it is oxidized in the liver, potentially yielding either two molecules or one molecule of vitamin A, depending upon whether the provitamin is  $\beta$ -carotene or one of the others, respectively. Recent work, however, has shown that man, as well as the experimental animal, is an inefficient converter of carotene to vitamin A. On the average, about four times as much carotene is required as vitamin A to maintain normal dark adaptation in adults, and in some cases there are even more marked individual variations. One would expect one molecule of  $\beta$ -carotene to yield two vitamin A molecules, but since it is only about half as active biologically, the  $\beta$ -carotene molecule is apparently split in an unsymmetrical manner. The other carotenoid precursors of vitamin A are even less active biologically since each molecule can form only one of vitamin A. This inefficiency of conversion may not be real but may be due to the fact that carotene is not absorbed as easily as is vitamin A and a considerable amount is lost in the feces. It apparently requires the presence of bile salts and of fat in the intestine for its absorption, whereas bile does not appear to be necessary for the absorption of vitamin A. Consequently, in cases in which there is a stoppage of bile, bile salts or desiccated bile should be administered in order to be sure that the provitamin is taken up. Another practical point is the fact that carotene is soluble in mineral oil. This may also be true of vitamin A (Steigmann). It has been shown that if mineral oil is taken shortly after a meal, it may remove much of the carotene present in the digesting food, and thus cause a deficiency of the vitamin. For individuals using mineral oil constantly this is a danger which should be recognized. Whether bile is required for the absorption of vitamin A is a matter of dispute. Apparently the presence of fat is not a prerequisite, but bile salts are helpful if not essential. Vitamin E seems to have a sparing action on vitamin A. (See page 274.)

Neither vitamin A nor the provitamins pass the placenta into the fetus very readily, although the vitamin is more easily transferred. Consequently newborn infants have low stores of both. The milk of well-fed mothers contains ample amounts of this vitamin for the infant's needs.

**Effect of Deficiency.**—In the experimental animal a lack of vitamin A is manifested by a slowing or stopping of growth in the young. This effect, however, is not peculiar to vitamin A, since lack of other vitamins or other nutritive factors has similar results. The outstanding result of vitamin A deficiency is

upon the eye. This is manifested in animals by an avoidance of light (photophobia) and by the occurrence of xerophthalmia and keratomalacia. Xerophthalmia is an eye disease characterized by drying of the eyes. The cells of the lacrimal glands become keratinized and stop secreting tears. The external surfaces thus become dry and have a dull appearance. Ulcers form; bacteria are not washed away; the eyelids swell and become sticky and scaly. Frequently there are bloody exudates and severe eye infections. If not treated in time blindness results, but in most instances the animals die of respiratory infection before this occurs. The reason for this is that vitamin A deficiency has an effect upon other epithelial structures as well as those of the eye. In other words, the eye affection is only one manifestation of the specific influence which this vitamin has upon many epithelial structures. This is "the substitution of stratified keratinizing epithelium for the normal epithelium in various parts of the respiratory tract, alimentary tract, eyes and paraocular glands, and the genito-urinary tract" (Wolbach). One of the results of this keratinization is the loss of cilia in the respiratory epithelium. These ordinarily tend to sweep upward bacteria-laden foreign particles, and thus combat infection. In rats, vitamin A is definitely necessary for reproduction and lactation. In fact it is just as essential as vitamin E, the "antisterility" vitamin, and must be given in greater amounts than are needed for optimal growth, if normal reproduction and lactation are to occur.

In man, deficiencies in vitamin A result in epidermal lesions and ocular changes. The appellation, "antiinfective" vitamin, which was formerly given to this vitamin, is not justified. As stated, a lack of the vitamin may contribute to infection, and there is no doubt that a lowered resistance is brought about.

Extreme cases of vitamin A deficiency in man are very rare at the present time in Western civilization, although in Eastern countries it is still seen. Livingstone's party suffered from it in 1857 in their African explorations as a result of a diet of coffee, manioc roots, and meal, and there have been many instances described since. In 1904 there was a report of 1,400 cases of xerophthalmia among Japanese children. During and after World War I many cases of the same condition occurred in Denmark, because of the fact that butterfat was shipped out of that country in large amounts and substitutes containing no vitamin A were eaten. Xerophthalmia, of course, results from a total or nearly total lack of vitamin A, and it is seldom that a person today subsists on a diet of that type.

Less serious symptoms are frequently found in human beings because of a diet containing less A than the required minimum. Night blindness, nyctalopia, is often encountered. This is an inability to see in dim light or to adapt to a decrease in intensity of light. Both the rods and the cones of the retina contain substances which depend upon vitamin A for their formation and regeneration. The rods are particularly concerned in dark adaptation, and the vitamin is especially needed for this function. Night blindness is frequently associated with cirrhosis of the liver, according to Haig and Patek, and with other liver conditions, which may indicate that the liver has something to do with the activity of vitamin A, although it may simply be related to the storage



and absorption of the vitamin. The most important chronic disease in which carotene cannot be transformed easily into vitamin A is diabetes mellitus. If diabetic subjects are on restricted diets without insulin and try to satisfy their hunger with large amounts of green and yellow vegetables, their skin may acquire a yellow tinge due to the deposition in it of carotene. Night blindness is likely to occur under such conditions, but the addition of the vitamin itself to the diet quickly brings a return to normal vision. Perhaps even earlier than night blindness is the occurrence of xerosis conjunctiva, minute dry spots which may be detected by biomicroscopic examination. Kruse believes this condition to be quite



Fig. 34.—Follicular hyperkeratosis as seen in some cases of severe chronic vitamin A deficiency. Area of skin on thigh shows follicular papules with projecting horny spines and hyperpigmentation. (From Frazier, C. N., and Hu, C. -K: *Arch. Int. Med.* 48: 507, 1931.)

prevalent in the population of this country, due to an inadequate intake of vitamin A. Both night blindness and the other eye symptoms are treated by administration of carotene or, better, of vitamin A. Fairly large doses are given, but there is a limit to the amount which can be absorbed, or put to work, in healing the damage present. Usually results are noted in a very short time. It has recently been shown that, when administered in water-soluble vehicles, vitamin A is effective in much smaller dosage than in an oily or fatty medium.

Skin conditions frequently result from an inadequate vitamin A intake. Dryness and scaliness of the skin are often seen as early stages of vitamin A deficiency. Sometimes small pustules appear around the hair follicles on extensor surfaces of the upper and lower extremities, on the shoulders, neck, back, lower abdomen, and buttocks. They are hard and pigmented and are surrounded by a zone of pigmentation (Fig. 34). In other instances the pimples resemble those of acne except that there is seldom any pus. Large doses of vitamin A are required over a period of many weeks to cure these conditions. Although the epithelium of the mucous membranes is often keratinized in animals, it is not certain whether similar pathological changes occur in man. Another finding in animals with A deficiencies is urinary calculi, and here again it is doubtful whether the same result follows in man.

Vitamin A is an important factor in tooth formation. This is probably related to the fact that the enamel layer is an epidermal structure. As a result of vitamin A deficiency, there is a defective formation of enamel with the consequent exposure of the dentine. Sound teeth, of course, cannot be expected under such circumstances.

Vitamin A deficiency has been asserted to result in paralysis and nerve degeneration. All authorities do not agree on this, however. The explanation may lie in the fact that such a deficiency may retard bone growth and, in particular, the formation of endochondral bone, while the central nervous system, as well as other soft tissues, continues to grow at a nearly normal rate. If this occurs at a very early age it has an effect upon the nervous system. As the skull does not grow rapidly enough, there may be overcrowding of the cranial cavity with distortion of the brain and pressure upon the spinal cord and nerve fibers (Wolbach and Bessey). Therefore, the nervous lesions may be entirely mechanical in origin. These results have been seen to occur in laboratory animals, but whether they occur in man is not certain.

The retardation of endochondral bone formation must be specific because bone matrix (osteoid) formation continues. In fact, the bony labyrinth of the ear of laboratory animals is subject to an overgrowth of bone.

Other effects which have been attributed to an avitaminosis of this vitamin are atrophy of the testes and disturbances of the female genitals.

**Mode of Action.**—Although the consequences of vitamin A deficiency in many parts of the body are known, its mode of action is not well understood except in relation to the retina, where it plays a direct role in the chemistry of vision.

The light receptors of the eye are the retinal rods and cones. Both kinds of receptor contain light-sensitive pigments, which require vitamin A for their formation and proper functioning. The pigment contained in the rods, *visual purple*, or *rhodopsin*, is a conjugated protein in which the prosthetic group is a red-colored carotenoid. Visual purple is extractable from the rods with mild detergents, such as bile salts or digitonin, with which it forms a soluble complex, but is insoluble in the usual protein solvents. Its molecular weight lies between 270,000 and 800,000. It is very sensitive to light and, when illuminated,

nated, changes from red to orange to yellow and, on prolonged exposure, to colorless vitamin A, and protein. The eye becomes less sensitive to light during the bleaching of rhodopsin (light adaptation). In the dark, rhodopsin is regenerated and the sensitivity of the retina is restored (dark adaptation) in exact proportion to the concentration of rhodopsin regenerated. The recovery is rapid or slow, according to the intensity and duration of the pre-exposure to light. If there is a deficiency of vitamin A in the retina, regeneration is incomplete, and the patient is said to be night blind.

Regeneration of rhodopsin in the test tube was first reported in 1879 by Kühne, and only recently rediscovered (Hecht). The chemical details, subsequently investigated, may be summarized as follows (Wald):

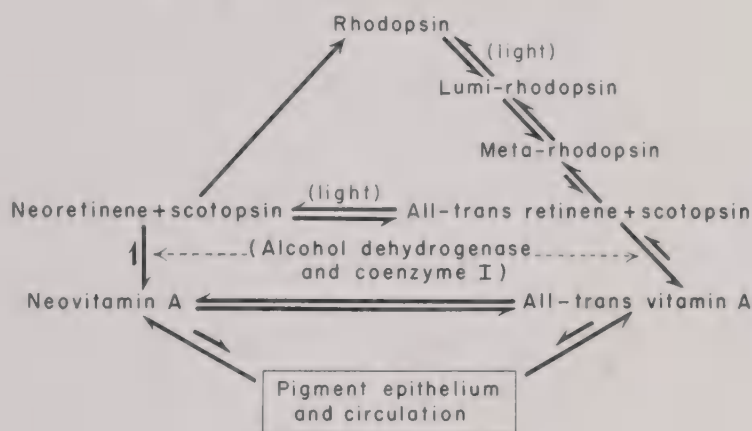


Fig. 35.—Probable transformations occurring in the retina during the visual cycle. (Courtesy of Dr. Charles Haig.)

In the bleaching of rhodopsin by light, the first step is entirely a light process, producing *lumi-rhodopsin*, which is orange-red in color. Subsequent steps require no light. The second step converts lumi-rhodopsin into orange *meta-rhodopsin*. These changes are associated with the exposure of -SII groups on the protein fraction, called *scotopsin*. The -SII groups attract positive ions and, presumably, act as cathodes to excite the rod cells, thus leading to excitation of the associated nerve fibers and we "see" the light. In the dark, most of the meta-rhodopsin is rapidly reconverted to lumi-rhodopsin and rhodopsin (fast dark adaptation). Some, however, decomposes to produce yellow *all-trans retinene* (vitamin A aldehyde) and scotopsin. If the light is still illuminating the eye, some of the trans retinene is changed to the cis form, *neoretinene* or *cis retinene*, while the remainder is reduced to colorless *all-trans vitamin A* (vitamin A alcohol). The latter step, which will also occur in complete darkness, is catalyzed by alcohol dehydrogenase and coenzyme I. All of these reactions are reversible (dark adaptation), and, in addition, all-trans vitamin A is supplied by the blood via the pigment epithelium to replace losses through displacement. There are also trans-cis reactions producing, reversibly, *neoretinene* and *neovitamin A* (cis vitamin A). In addition, rhodopsin is slowly re-formed in the dark directly from neoretinene (slow dark adaptation). These reactions are summarized in the scheme shown in Fig. 35.



It has been established by experiments on patients and normal controls that vitamin A is required by the cone pigments, as well as by rhodopsin (Haug). It is probable, therefore, that the chemical mechanisms of the cones are similar to those of the rods, even though the cones function in daylight whereas the rods function in dim light.

*Porphyropsin* is a purple light-sensitive pigment, extractable from the rods of vertebrates that start life in fresh water. The porphyropsin system is entirely analogous to the rhodopsin system but employs vitamin A<sub>2</sub> instead of A<sub>1</sub>. The absorption spectra, and therefore the colors, of porphyropsin and its derivatives are all shifted toward the red end of the spectrum. On the other hand, the proteins of porphyropsin and rhodopsin are probably the same.

**STORAGE.**—Most of the carotene and vitamin A absorbed goes to the liver where it is stored. It is possible to hoard a sufficient amount of this vitamin to last a long time. The method whereby it is released from the liver for physiological use is not known.

**EXCRETION AND SECRETION.**—Neither vitamin A nor provitamin A is excreted in the urine. They may appear in the feces but probably this is an unabsorbed portion. Even this happens to only a slight extent, the unused material being destroyed by enzymes or bacteria. Amounts in liver and other tissues in excess of the normal storage capacity or requirement must also be destroyed but how this happens is still unknown. Both carotene and the vitamin are secreted by the mammary gland. Human colostrum has two or three times as much as early human milk, and the latter has from five to ten times the vitamin A activity of cow's milk.

**Human Requirements.**—In measuring this, as well as the other vitamins certain "units" have been used. These were at first rather arbitrarily fixed and were often based upon the amount necessary to prevent avitaminosis from occurring in animals under standard conditions. In time, as the vitamins were synthesized, it became possible to base the unitage upon the weight of carefully purified and standardized preparations. For vitamin A the World Health Organization has chosen as one international unit the activity of 0.000344 mg. (0.344  $\mu$ g) of synthetic vitamin A acetate, which is equivalent to 0.300  $\mu$ g of vitamin A alcohol.

The minimum daily allowance of vitamin A recommended is about 5,000 units for both adults and growing children. (See Table XXIX, page 326.) For pregnant and nursing women from 6,000 to 8,000 units daily are recommended. The well-balanced dietary of most Americans contains this amount under normal conditions, but it is more than possible that the underprivileged do not get the minimum. It is also possible that some individuals require more than the minimum either because of faulty absorption or for other reasons. Therefore the addition of supplements may be indicated, especially since a moderate excess seems to be nontoxic.

### Vitamin D

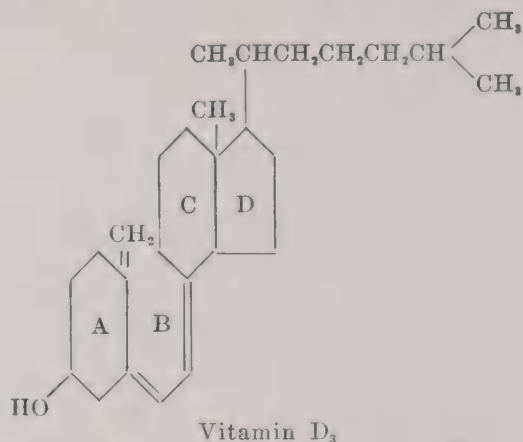
Vitamin D is the vitamin which is related to rickets and is therefore spoken of as the "antirachitic" vitamin. It is necessary for normal calcium and phosphorus metabolism, and therefore for healthy bone and tooth development.

**Discovery and Properties.**—In the very early days of the vitamins, “fat-soluble A” was considered a single substance, the vitamin which was capable of curing both xerophthalmia and rickets. In 1922 McCollum bubbled oxygen through cod-liver oil for hours at 120° C. and found that the resulting oil was ineffective against xerophthalmia but was capable of curing rickets. Vitamin A had been destroyed by this procedure, and the factor remaining was called vitamin D, the antirachitic factor. Other evidence was brought forward to prove the same point. For example, it was found that a very small amount of dehydrated spinach would cure xerophthalmia in rats in a few days but huge amounts of the same dried spinach could not prevent rickets when rats were fed a rachitic diet (McClendon and Schuck.) Vitamin D is a white crystalline substance, soluble in fats and fat solvents. It is evident that it is heat resistant and also resistant to oxidation. It is also not affected by acids or alkalis. When cod-liver oil is saponified, the vitamin is found in the nonsaponifiable fraction. It is a sterol.

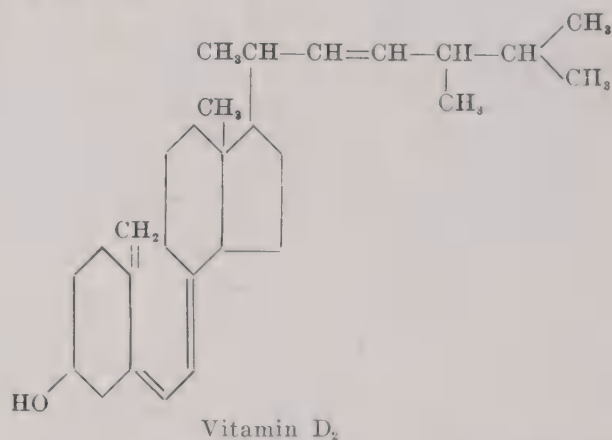
**Relation to Radiant Energy.**—From the time of Herodotus, the health-giving effects of sunlight have been emphasized, and the particular relationship of lack of sunshine to rickets began to be recognized in the last century. It was seen that underprivileged children, living in dark, crowded quarters, were likely to be victims of this disease. There was a greater incidence in winter than in summer, and this was considered to be due to the differences in the length of the days. In 1822 a Polish physician, Sniadecki, maintained that exposure of the body to direct sunlight had both a preventive and a curative effect for rickets. Much later, Palm, an Englishman, as a result of correspondence with medical missionaries all over the world, revealed the absence of rickets, in spite of poor sanitary conditions, in those regions where sunlight was most abundant, and definitely concluded that rickets was mainly caused by a lack of sunlight. However, demonstration of the curative action of ultraviolet rays by modern methods of x-ray diagnosis were lacking. In 1919 Huldshinsky proved by such methods that exposure to ultraviolet rays would cure rickets, and Hess duplicated this success with sunlight. Later it was shown that animals fed on a diet deficient in vitamin D but containing a sufficiency of calcium and phosphorus would not acquire rickets if they were irradiated with ultraviolet light. The final chapter in this story was the announcement that cholesterol-containing inactive foods could be given antirachitic properties by irradiating them with ultraviolet light (Hess and Steenbock). Dried milk and other foods can be irradiated on a commercial basis. A potent antirachitic oil is produced by the irradiation of ergosterol, a sterol derived from ergot and from yeast. Irradiated ergosterol is known pharmaceutically as “viosterol.”

The explanation of these phenomena is that ultraviolet irradiation causes a change in the molecular structure of some sterols which, unless so changed, are inactive. If the irradiated sterols are taken by mouth they act just the same as the naturally occurring vitamin D; if they are produced in or on the skin by the irradiation of the inactive sterols present there, they are absorbed and find their way into the circulation and behave similarly to the orally administered vitamin. These inactive sterols are the provitamins of vitamin D. One of them, 7-dehydro-cholesterol, can be synthesized by animals, including man.

**Structure.**—At least ten different compounds are known to have anti-rachitic properties and are designated  $D_1$ ,  $D_2$ , etc. Five of them are rather well defined as chemical compounds, but only two are of great importance,  $D_2$  of vegetable origin and  $D_3$ , of animal origin. The common vitamin D of fish liver oils is  $D_3$ . This is probably the form also present in milk and eggs and is produced on irradiation of the skin and when 7-dehydrocholesterol is irradiated. The formula shows that the effect of irradiation is the opening of ring "B."



It should be stated that 7-dehydrocholesterol differs from cholesterol only in having two less hydrogen atoms, thereby introducing a second double bond, also in ring "B." Vitamin  $D_2$  is also known as calciferol and is the one mentioned as being derived from ergosterol by ultraviolet irradiation. The formula of this is strikingly similar to that of  $D_3$ .



Both forms of the vitamin have about the same degree of activity in the human being, although they are not equally effective in the chick. In nature these vitamins occur as esters.

**Occurrence.**—Cod-liver oil and other fish liver oils are the best sources of vitamin D that we have. The flesh of oily fish, such as sardines, salmon, and herring, are also excellent sources. Egg yolk and liver of the common slaughtered animals contain amounts which depend upon the food of the animal from which they are derived, but mammalian liver is not very rich in the vitamin. Milk contains little vitamin D unless enriched in one of the three ways



described in Chapter 7, and "vitamin D milk" is now a common article of diet. Many of our ordinary foods, among them the green plants, contain very small quantities, and mushrooms contain slightly greater amounts. In general, this vitamin is not very widely distributed, but the fact that it can be provided in three ways should make its deficiency rather uncommon in the future. These three ways of providing vitamin D are, of course, (1) by furnishing the vitamin as it occurs naturally in foods, (2) by irradiating foods containing precursors of the vitamin, and (3) by irradiating, with ultra-violet light or sunshine, the skin of the individual.

*a**b*

Fig. 36.—Rachitic children. *a*, Knock-knees; *b*, bowlegs. (From Brennemann, J.: Practice of Pediatrics, Hagerstown, Md., 1944, W. F. Prior Co., Inc.)

**Absorption.**—Absorption of the vitamin from the intestinal tract requires the presence of bile. Here, again, mineral oil acts as a hindrance, because the vitamin is oil soluble and consequently goes through the intestine into the feces with it. As stated above, irradiation of the skin with ultraviolet light results in the formation of vitamin D. This appears to occur on the skin rather than in the skin, and absorption follows from the surface. Helmer and Jansen found that the grease washed off the bodies of irradiated individuals had antirachitic properties, while that from men who had not been irradiated had very little potency. It would seem, therefore, that swimming after sunbathing is less physiological than sunbathing after swimming.

**Effects of Deficiency.**—A deficiency of vitamin D leads to rickets in children (Fig. 36) and to osteomalacia in adults, a condition which might be termed

adult rickets. Rickets usually develops in infancy or early childhood, although juvenile or late rickets is seen in India and x-ray-detectable rickets is frequently observed in that country up to the age of puberty. Defective ossification is the result of this avitaminosis; the bones become soft and pliable and a number of different deformities may ensue: bowlegs, knock-knees, enlargement of the ends of bones (epiphyses), rows of beadlike swellings at the rib junctions (the "rachitic rosary"), contracted pelvis, and the development of bosses on the temporal bones. X-ray photographs of the bones reveal that ossification is not normal; the shadows cast are less dense and the ends of the bones less sharply defined (Fig. 37). Chemical analysis of the bones reveals a low content of inorganic constituents and a high content of organic substances and water. However, the ratio of calcium to phosphorus remains constant, indicating that the type of bone

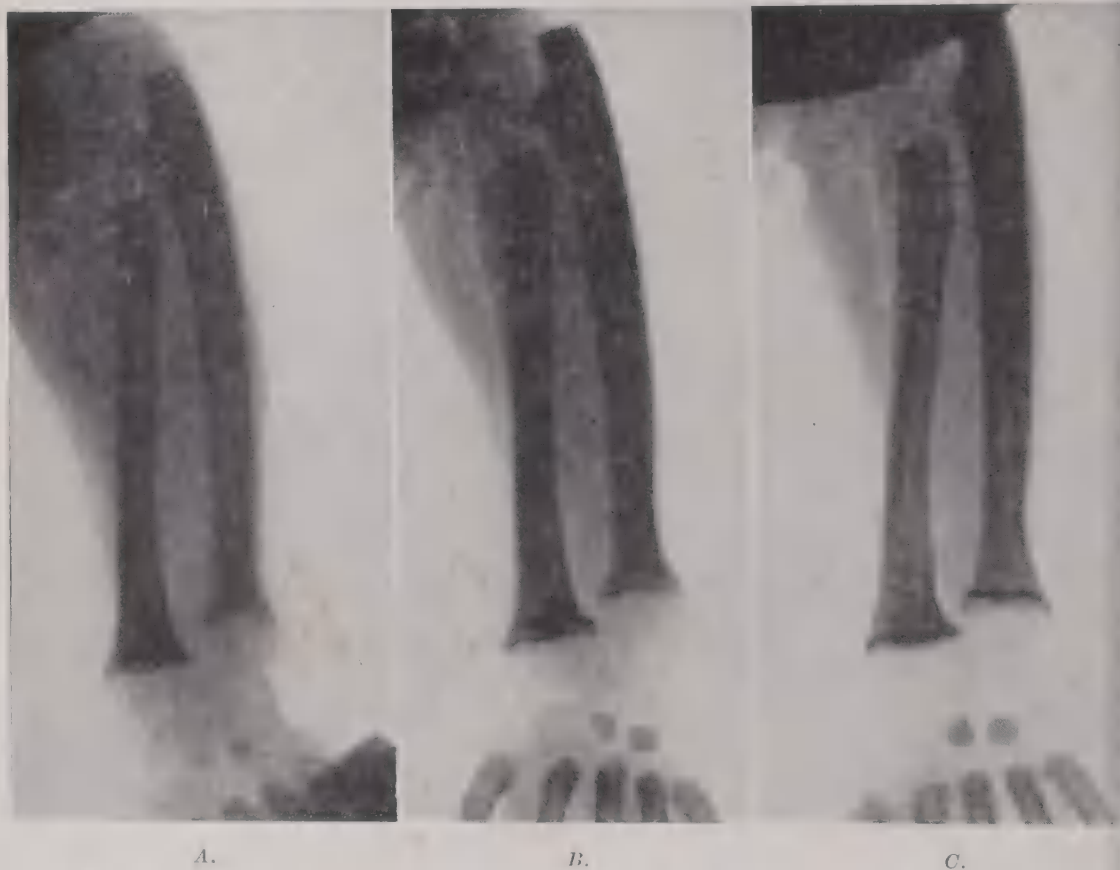


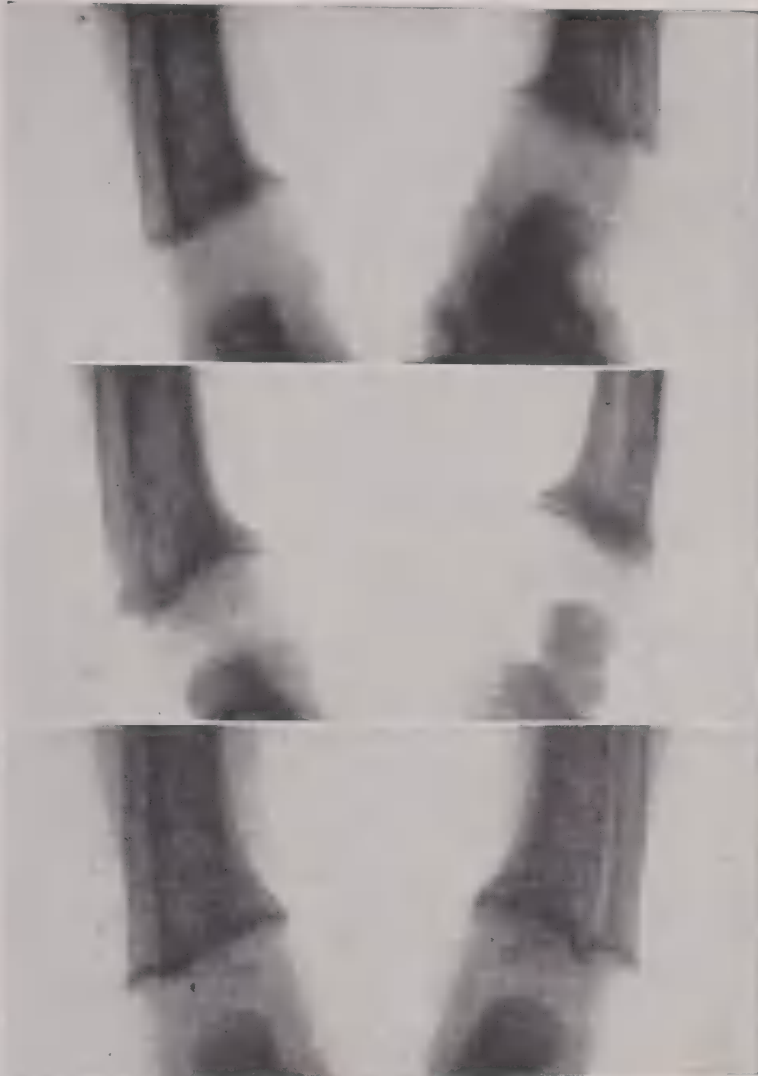
Fig. 37.—X-rays of the forearms and legs of a rachitic child, showing the effect of treatment.

A, B, and C, x-rays of the forearm.

A, Feb. 11, 1938, marked trabeculation of the radius and ulna, particularly in the cortical portion of the bone with slight periosteal thickening. The end of the bone is slightly mushroomed and cupped and shows distinct fringing. The cartilage is swollen. The distal epiphyses of the radius are absent. There are two small centers of ossification in the wrists.

B, March 11, 1938, a new center of ossification has appeared in the distal epiphysis of the humerus. The centers of ossification at the wrists are still two in number but larger and more distinct in outline. There is a fresh line of calcium deposition at the ends of the radius and ulna in the zone of provisional cartilage (line test). A clear area is present between this new calcification and the shaft (submetaphyseal rarefaction). Cupping, fringing, and stippling are present and spur formation is also noted outlining the swollen cartilage at the distal epiphysis of the ulna (beginning healing).

C, March 24, 1938, healing is now advanced. The shaft shows calcification of subperiosteal osteoid at the outer aspect of the radius, giving the bone a greater width. Calcification of the provisional zone in the metaphyses is much more distinct and the submetaphyseal area is filling in. (Continued on opposite page.)



D.

E.

F.

Fig. 37 (cont'd).—D, E, and F, x-rays of distal ends of the tibiae and fibulae.

D, Feb. 11, 1938, cupping of the distal ends of both tibiae and fibulae with compression and widening of the end of the shaft.

E, March 11, 1938, beginning healing of the distal ends of both bones (tibia and fibula) (line test). Submetaphyseal rarefaction. Periosteal hyperplasia at the distal third of both tibiae.

F, March 24, 1938, advanced healing. Submetaphyseal rarefaction is now well filled. The ends of the bones are well calcified with marked stippling in the distal cartilage of the tibia and the beginnings of a center of ossification in the distal epiphyseal cartilage of the tibia.

*Case History.*—Patient I. B. E. Diagnosis: Rickets and infantile tetany. Admitted Feb. 9, 1938, because of carpopedal spasm of six hours' duration. Had never had cod-liver oil. Had 3 ounces orange juice daily for one month (6 months old upon admission). Physical examination on admission: Carpopedal spasm, separation of the sagittal and lambdoid sutures with marked occipitoparietal craniotabes. Thorax: Flaring of the lower ribs with enlargement of the costochondral junctions. Chvostek's sign, positive. Laboratory data: Serum  $\text{CO}_2$ , 1 vol. per cent: Ca, 4.6; P, 5.6 (mg. in 100 ml.); Ca  $\times$  P, 25.7; phosphatase, 57.2 units (Bodansky). Treatment: Calcium gluconate, 10 ml. of a 10 per cent solution, intramuscularly at 10 P.M. and at 12 P.M. Calcium chloride, 1 dram q. 4 h. (1 gram). Orange juice, 2 ounces daily. Drisdol, a vitamin D preparation, 10 att. daily; i.e., approximately 2,500 I.U. vitamin D. February 20, calcium chloride discontinued.

#### Blood Analysis

Date	Ca	P	Ca $\times$ P	Phosphatase
/9/38	4.6	5.6	25.7	57.2
/17/38	7.1	3.0	21	32
/28/38	8.6	3.7	32	17
/7/38	9.7	4.7	46	--
/10/38	9.4	5.9	55	--

Note particularly the relation of the Ca  $\times$  P values to the x-ray findings. (Photographs and case history courtesy Dr. Benjamin Kramer.)



salt laid down is normal, although the amount is insufficient. In the blood serum there is usually, but not always, a normal content of calcium, but the phosphate is decreased. Howland and Kramer, who first discovered this, stated that if the product of the serum calcium content and the serum phosphate, both expressed as milligrams per 100 ml., equalled or exceeded 40, rickets did not develop; whereas a product of below 30 always led to rickets. There is also a marked increase in serum phosphatase in rickets and a decrease when recovery is brought about as a result of vitamin D treatment. The exact significance of this is not known. Some believe that the increased serum phosphatase in rickets (and in other bone diseases) is a result of overproduction in the bone in a vain attempt at bone formation. Others consider it a result of the bone's increased capacity for cellular activities because of the absence of true bone which is relatively inactive.

In rickets there is commonly a delay in dentition. The first tooth in rachitic babies seldom appears between the sixth and ninth month, at which time it has appeared in about half of the number of normal babies. This would be expected in view of the close relationship of bones and teeth. Lady Mellanby has shown also that lack of vitamin D leads to hypoplasia of teeth in dogs; that is, poor structural development. This may predispose to dental caries since a hypoplastic tooth is less effectively protected by enamel. The question as to whether healthy human teeth are likely to be more carious if vitamin D is lacking is still unsettled, as is also the companion question of the effect of a high vitamin D intake in preventing caries. Much work has been done on both of these problems, and results indicate that vitamin D probably reduces the incidence of caries indirectly; that is, by improving the general health and nutrition of the individual.

Osteomalacia presents a somewhat different picture from rickets, although the action on the bones is essentially the same. The bone becomes softer than rachitic bone and the ratio of calcium to phosphorus is changed. The loss of calcium is greater than that of phosphorus, and there is a relative gain in magnesium. This softness of the bones leads to diverse types of deformities. Osteomalacia occurs very rarely in America or Europe, except in old age, but is common in India and China, particularly among the women because of the customs which confine them indoors. Thus they are deprived of exposure to sunshine, and their diet is also lacking in foods containing vitamin D and calcium. In osteomalacia the serum calcium is reduced sometimes to such an extent that tetany ensues. Tetany is a state of muscular twitching which is brought about by low blood calcium.

Another clinical condition indirectly associated with a lack of vitamin D is celiac disease, also known as idiopathic steatorrhea and nontropical sprue. Here, as in osteomalacia, there is a demineralization of the bones. This may result in deformities or dwarfism. Here, too, a low serum calcium and low serum phosphorus are found, with possible manifestations of tetany. It is *indirectly* a vitamin D deficiency because the primary abnormality seems to be fatty diarrhea. The fat in the intestinal canal is not absorbed normally and is carried with it into the stools as calcium salts (soaps) and vitamin D.

In all of the conditions mentioned, the administration of the vitamin in therapeutic doses, or ultraviolet irradiation, or both, produces good results. Deformities cannot be rectified by this means, but further malformation may be checked. Serial x-ray photographs of rachitic bones before and during treatment show the effect in a striking manner. (Fig. 37.)

**Mechanism of Action.**—Vitamin D has a regulatory power on calcium and phosphorus metabolism. Both calcium and phosphorus must be present in the diet in order to have the complex calcium salt deposited in bone. However, no matter how great an amount of these minerals is available, normal calcification will not take place in the absence of this vitamin. On the other hand, if the supply of calcium and phosphorus is practically at starvation levels, an optimum amount of vitamin D will enable them to be utilized and deposited in a nearly normal manner. "Vitamin D not only acts as a regulator of the metabolism of these elements; it permits the body to operate with greatly increased economy" (McCollum). It does this by apparently influencing two different functions. In the first place, it causes an increased absorption of calcium and phosphorus from the intestinal tract. The other action is a local one; it is indispensable for the actual deposition of bone. Besides the specific effect upon bone, vitamin D is said to have an influence upon the general metabolic condition of the body. A deficiency is accompanied by a low metabolic rate, which may be raised by the administration of the vitamin.

**TESTS FOR VITAMIN D.**—In experimental animals there are several tests for vitamin D deficiency and recovery. X-ray photographs of the distal ends of the ulna and radius show a characteristic indistinctness. When healing occurs, the entire bone assumes a more homogeneous appearance and the cupped ends fill out. The "line test" is very useful in experimental studies when it is necessary to ascertain, on autopsy, the degree of healing. The split bone is treated with silver nitrate. This stains the provisional zone of recalcification, leaving the uncalcified rachitic tissue unstained. Another method is to analyze the bone for total ash. In rickets, the total ash is low, although the Ca:P ratio remains normal.

**Human Requirements.**—The requirements of normal infants and children will depend partly upon the amount of ultraviolet light to which they are exposed. It should be remembered that the effective ultraviolet rays do not penetrate ordinary glass. Therefore, exposure to sunshine coming through window glass is of no value. Smoke also impedes the progress of these rays and consequently city sunshine is not always beneficial. For this and other reasons, a rather wide "spread" in the recommended amount is suggested. This is 400 to 800 International units for infants. The same amount is advised for women in the latter half of pregnancy and during lactation. For other adults and for older children probably 400 units is sufficient.

After administration of an excess of vitamin D to a mammal, it can be found in the circulating blood for months. It is undoubtedly stored in several organs but not to a great extent in the liver, as is the case in fish. Any excretion is by way of the bile. Since the distribution of this factor in foods is quite uncertain and exposure to sunshine is often inadequate for long periods of





organic compounds, some of them quite unrelated structurally to the vitamin. Hundreds of compounds, phenols, quinones, coumarins, etc., show more or less vitamin E action. Those which are similar structurally are called "vitamers"; that is, substances not occurring naturally which are similar in structure and activity to the natural vitamin. On the other hand, slight changes in the structure of the active tocopherols, such as shortening the side chain, may reduce or even abolish its physiological effects.

**Absorption.**—Although there is little evidence regarding the manner in which vitamin E is absorbed, it is believed that it is similar to the other fat-soluble vitamins in this respect. Bile salts and the presence of fats are thought to be useful if not entirely essential. However, rancid fats destroy this vitamin by oxidation (see page 82).

If the mother is on an adequate diet, the fetus absorbs through the placenta sufficient tocopherol for its needs, but not enough for storage. This must be supplied to the young animal (and presumably to the infant) by the milk. Storage occurs in various tissues (active mammary tissue, liver, heart, lungs, spleen, adipose tissue, and muscles), but authorities disagree as to the relative capacities of the different organs.

Water-soluble forms are available. Eppstein and Morgulis found that the water-soluble disodium phosphate ester of  $\alpha$ -tocopherol, when administered to rabbits intramuscularly, had a more rapid and constant effect than the oil-soluble vitamin, administered orally.

**Effects of Deficiency.**—In rats a lack of vitamin E results in damage to the reproductive system of both males and females. There is a degeneration of the germinal epithelium which cannot be remedied, after it is once established, by feeding the vitamin. If the female on a vitamin E-free diet does become pregnant, the embryo dies and is resorbed. In mice, deprivation of vitamin E does not cause testicular degeneration in the male, but in pregnant females the same resorption of the fetus takes place as in rats (Bryan and Mason).

At the present time it cannot be definitely stated that man requires vitamin E for the reproductive functions. Many clinical investigations have been reported in which vitamin E concentrates were used to attempt to remedy sterility and habitual abortion, but it is now rather generally agreed that vitamin E is of no value in these conditions.

Besides the effects on the reproductive system, a lack of vitamin E has been found to cause atrophy of the voluntary muscles (muscular dystrophy) in several species of laboratory animals. In young mice, born of mothers deprived of vitamin E (after the inception of pregnancy), edema of the subcutaneous and intramuscular connective tissue is found, and in some cases necrosis of skeletal muscle fibers (Pappenheimer). If the animals survive, spontaneous cure takes place, even though vitamin E is withheld. In some species this is not the case; the disease is progressive and continues into adult life. In chicks the deficiency results in an injury to the nervous system due to an impairment of the blood vessels in the brain. Here again there seems to be no comparable effect upon human beings. In rabbits it has been found that nucleic acid metabolism is

deranged. This is shown by a higher output of allantoin and a change in the content of tissue nucleic acids. (Young and Dinning.)

Vitamin E has a sparing action on vitamin A and carotene. For example, vitamin A and carotene are more effective in curing their deficiency symptoms if E is administered at the same time. Ingestion of extra amounts of  $\alpha$ -tocopherol increases the storage of A in the liver of rats, and many other examples of the close connection between these two vitamins could be cited. This biological relationship undoubtedly has a chemical basis. Vitamin E is an antioxidant; that is, it can prevent the oxidation of various other easily oxidized substances, notably fats (see page 83) and vitamin A. For that reason it is often added to foods to prevent oxidation. Vitamin A is rather easily oxidized, and these tocopherols have an antioxidant action toward it also. Possibly this protection is effective even within the cells. Vitamin A, it will be remembered, is also essential for reproduction. Now, although the beneficial action of vitamin A is primarily upon ectodermal and endodermal tissues and that of E is on the mesoderm, it is quite likely that E, by preventing the too rapid destruction of A, indirectly influences all three layers of the embryo.

Bicknell and Prescott feel that the basic action of the tocopherols is to inhibit oxidative processes in all tissues. The tissues of a young rat, suffering from E deficiency, have an increased capacity for taking up oxygen before any signs of muscular dystrophy occur. Injections of  $\alpha$ -tocopheryl phosphate into such animals cause the tissues to acquire an almost normal degree of oxygen consumption. The antioxidant properties of vitamin E are enhanced by certain other substances, many of which are also antioxidants. Phenols and ascorbic acid are notable examples.

Shute found that vitamin E administration is useful in the treatment of purpura (subcutaneous extravasation of blood). While studying this phenomenon, he noticed an improvement in the heart condition of some of his patients. This led to treatment of cardiac and other vascular diseases with vitamin E, with beneficial results. His results have not been generally confirmed.

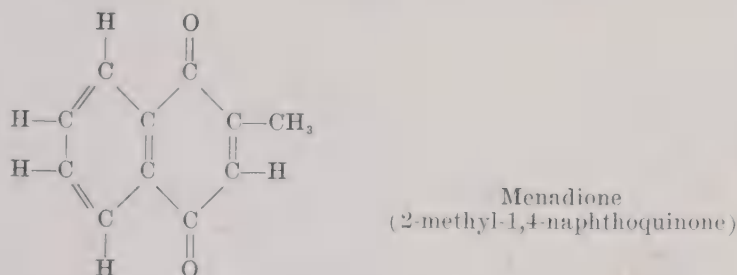
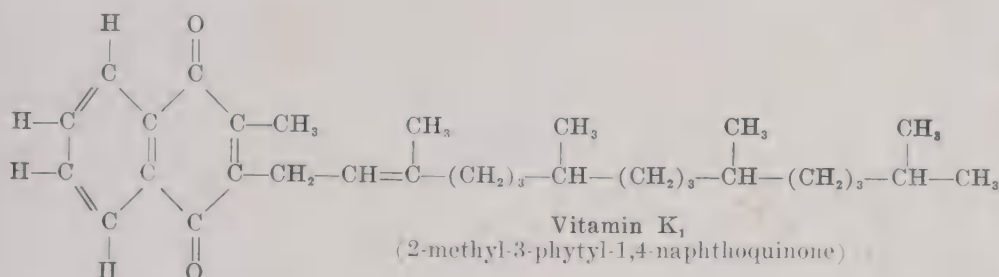
### Vitamin K

Dam, a Danish investigator, in 1934 discovered a hemorrhagic disease in chicks due to the lack of a food factor which he called "Koagulations Vitamin." From this came the term vitamin K. A deficiency of vitamin K leads to a slowing of the rate of blood clotting. More specifically, there is an increased "prothrombin time" (see page 191). It therefore appears that this factor is needed by the body for the production of a normal amount of prothrombin. It is a fat-soluble substance found in various food oils and is of immense importance from a medical and surgical standpoint.

**Properties.**—The naturally occurring vitamin K is fat soluble and stable to heat and to reducing agents. It should be kept in dark bottles since it is sensitive to light. The activity is also abolished by alkalies, strong acids, and oxidizing agents.

**Structure.**—Vitamin K<sub>1</sub>, obtained from the alfalfa leaf, is 2-methyl-3-phytyl-1,4-naphthaquinone. Vitamin K<sub>2</sub>, produced by bacterial synthesis, is

2-methyl-3-difarnesyl-1,4-naphthaquinone. Many vitamers, that is, synthetic products with similar structures, having antihemorrhagic effects, have been prepared. Some of these are water soluble but only one is more potent (weight for weight) than  $K_1$ . This is 2-methyl-1,4-naphthoquinone, which has been given the name *menadione*. This is soluble in oil, sparingly soluble in water, and stable to air when protected from light. Its diphosphate ester is water soluble and is widely used clinically.



**Occurrence.**—As mentioned, vitamin  $K_1$  was obtained from alfalfa. A rich source of  $K_2$  is putrefied fish meal. Since both of these natural types have the same general activity, there is no need to distinguish them. Other excellent sources are cabbage, cauliflower, kale, spinach, and other green vegetables. Good sources include tomatoes, cheese, egg yolk, and liver. It is also found in a number of bacteria and is undoubtedly synthesized by microorganisms in the intestinal tract and is available to the host.

**Absorption.**—Since the natural vitamins K are fat soluble, they require bile in order to be absorbed, and consequently absorption occurs in the upper parts of the small intestine where bile salts are present. A vitamin K deficiency is very likely to occur whenever bile is prevented from entering the intestinal tract. This is true of most of the fat-soluble vitamins, but it is particularly important in the case of K because of its bearing on blood clotting. Thus, when there is an obstruction of the bile channels and jaundice ensues, there is delayed clotting. This is not due to the occurrence of the bile in the blood, as was formerly thought, but to a deficiency of vitamin K, for the absorption of which the presence of bile is required. As in the case of fatty acids, it is probably the bile acids to which may be ascribed the specific function of absorption of fat-soluble vitamins. Consequently, whenever vitamin K is given per os, the presence of bile is essential, and if there is a deficient bile flow, a bile or bile salt preparation should be administered. The parenteral administration of one of the vitamers, menadione for instance, obviates this necessity. The water-soluble



analogues may be given orally without the use of bile or bile salts. Excess vitamin K can be stored, but it is not known in which tissues this occurs.

**Effects of Deficiency.**—Animals suffering from this deficiency have a remarkable tendency to bleed profusely from minor wounds, and slight bruises result in extensive subcutaneous hemorrhages. Blood withdrawn from such animals clots very slowly; in some cases it may remain fluid for hours. This is a result of lack of prothrombin. Newly hatched chicks on a vitamin K-free diet show a gradual diminution in the concentration of prothrombin in the blood. Their intestinal flora produces some vitamin K but not enough to prevent avitaminosis without additions from the diet. Administration of the vitamin brings the clotting time of their blood up to normal levels within a few hours. The vitamin does not form part of the prothrombin molecule but has some influence upon the production of prothrombin by the liver. Hepatectomized animals show a rapid decline in the blood-clotting power due to lowered prothrombin, and administration of vitamin K does not raise it. It should also be noted that vitamin K treatment is ineffective in the presence of a liver so badly damaged that it cannot produce prothrombin, or if the intestine is incapable of absorbing the orally administered vitamin.

In human beings the same effects are attributable to lack of vitamin K. In normal newborn infants the prothrombin level is low. It usually falls still further and reaches the minimum on the third day of life. This is undoubtedly due to a lack of vitamin K. Apparently the vitamin passes from the mother to the fetus with great difficulty, especially toward the end of pregnancy, and, since the intestine of the newborn infant is sterile, there is no opportunity for bacterial synthesis for a while. However, the prothrombin level may reach normal by the end of the first week, probably as a result of bacterial synthesis of vitamin K, concomitant with the ingestion of milk, and the establishment of the normal intestinal flora. The high incidence of hemorrhage in the newborn infant is thus easily accounted for, and hemorrhagic disease of newborn infants is a frequent cause of infant mortality. Often an intracranial hemorrhage may result in brain injury and, if the infant survives, imbecility or some other mental and nervous condition may result. Prophylactic treatment is recommended by many clinicians. The expectant mother is given vitamin K supplements for several days before delivery is expected. If this has not been done, the infant is given such treatment soon after birth.

In adults, it should be emphasized, there is seldom a lack of *available* vitamin K. It is either present in the food in sufficient quantity or is produced by bacterial activity in the intestinal canal. A deficiency in the system may usually be referred to one of three fundamental causes: (1) There may be faulty absorption of the vitamin due to lack of bile in the intestine because of an insufficient secretion of bile salts, obstruction of the bile duct, or intestinal lesions, obstruction, or surgical procedures in the intestine. (2) This and other fat-soluble vitamins may be swept into the feces, particularly if the intestinal contents are unusually greasy. This has been experienced in diarrheal diseases such as ulcerative colitis, sprue or celiac disease, or following excessive use of mineral oil. (3) The administration of sulfaguanidine, sulfasuxidine, or other

intestinal antiseptics may cause a deficiency by a limitation of the production of vitamin K by the intestinal flora. The surgeon, about to operate upon a patient known to have any of the conditions mentioned, will give vitamin K before the operation in order to avoid excessive hemorrhage during or after the operation. Bile or bile salts may be irritating and produce vomiting; therefore, instead of the natural vitamin, the water-soluble substitutes may be given by mouth, or these or some of the others may be given parenterally. It should be remembered that vitamin K is not always indicated when there is prothrombin deficiency, which may also result from various liver diseases which render the liver incapable of producing prothrombin. Intake of vitamin K cannot restore this function.

**Human Requirements.**—It is very difficult to produce a deficiency of vitamin K in mammals or human beings by dietary methods alone. As mentioned, there is no lack of vitamin K available. Consequently no standard requirement can be set.

**Antagonists.**—The effect of heparin and dicumarol in inhibiting blood-clotting was discussed on page 193. They act as antagonists to vitamin K since their action is to diminish the amount of available prothrombin. The salicylates also are antagonistic to vitamin K. Consequently when any of these are administered over a long period of time, supplements of the vitamin may be required to enable the liver to restore the prothrombin level to normal.

**Antistiffness Factor.**—A relatively new fat-soluble vitamin is the “antistiffness factor.” (Bahrs and Wulzen.) Its absence causes stiffness of the “wrists” and “elbows” of guinea pigs. The muscles atrophy and become streaked with bundles of fine white lines of calcium deposits. Calcium phosphate deposits are found under the skin, in the joints, and elsewhere. Cod-liver oil accelerates the onset of the condition and intensifies it. The vitamin is found in fresh kale or alfalfa and in fresh cream. It has now been isolated in crystalline form, the administration of which, in doses of 0.1 microgram per day for five days, cures the condition. (van Wagtendonck and Wulzen.) It has now been identified as stigmasterol. (Kaiser and Wulzen.)

## THE WATER-SOLUBLE VITAMINS

The individual water-soluble vitamins bear no closer resemblance to each other chemically than the fat-soluble vitamins. However, it is convenient to group them together merely because of their solubility. It was this characteristic which was the first basis for classification. It also becomes of some importance from nutritional and clinical aspects. That is, vitamin deficiencies are likely to be multiple in nature and often may be mixed ‘fat-soluble vitamin deficiencies or mixed water-soluble vitamin deficiencies. The water-soluble vitamins include ascorbic acid, thiamine, niacin, riboflavin, pyridoxine, pantothenic acid, biotin, p-aminobenzoic acid, pteroylglutamic acid, and vitamin B<sub>12</sub>. Inositol and choline are frequently included in this list, but it is felt by many nutritionists that they are not true vitamins, although deficiencies of them in the diet of experimental animals cause characteristic symptoms to develop.



However, both normally occur in animal tissues in amounts greater than are generally associated with vitamin values. Neither of them appears to be a coenzyme nor a part of one. They have been discussed in other sections.

### Vitamin C

Scurvy was probably the first disease to be definitely associated with a food deficiency. It was common in Europe in the fifteenth century and must have been known long before that. It frequently occurred among sailors on long voyages when fresh food was not available. An instance of this was the voyage of Vasco da Gama around the Cape of Good Hope. He lost more than half of his crew from scurvy. In 1535 Jacques Cartier's men were stricken with this disease during their explorations in Canada. Several died and the remainder were saved by drinking an extract of the leaves of an evergreen tree, as instructed by a friendly Indian. Scurvy was long the dread of Arctic explorers who had to provide food for months in advance and were, of course, unable to take fresh foods. It is said that Dutch mariners, as early as the fifteenth century, knew of the efficacy of fresh vegetables and citrus fruits in the cure of this condition. In 1747 Lind, a British naval surgeon, treated a number of scurvy sailors on his ship with different medicaments, including fruit juices. He observed dramatic recovery in those partaking of lemons and oranges and recommended lemon juice as a standard part of the ration. It was not until 1795 that this advice was heeded and the government had limes put into the ration of the British sailor. From this is derived the sobriquet "limey." In 1843 Pereira referred to lemon juice as "one of our most valuable antiscorbutic foods."

In scurvy there occur anemia, pains in the joints and hemorrhages from the mucous membranes of the mouth and gastrointestinal canal, skin (Fig. 38), muscles and subperiosteal tissues. The gums are particularly affected, showing swelling, redness, ulceration, and even gangrene (Plate I). Weakness and emaciation are seen in later stages. There are definite defects in skeletal calcification without much disturbance in mineral metabolism. For example, x-ray examination of the bones in scurvy shows a white line on the outside of the shaft, a line not seen in normal bone. The pathological change leading to all of these symptoms is a weakening in the endothelial wall of the capillaries, *because of a reduction in the amount of intercellular substance*. The body normally produces intercellular material, absorbs it, and replenishes it continually. It is this new formation of cementing and supporting material which does not occur in the absence of vitamin C. This deficiency in supporting material may extend to the cartilage, bone, muscles, and other tissues and is responsible for the symptoms mentioned. Vitamin C is essential for the production of intercellular material. For this reason it is necessary for the healing of wounds.

**Occurrence.**—From a nutritional standpoint, the citrus fruits and tomatoes are the best sources of vitamin C, the antiscorbutic vitamin (now usually called ascorbic acid). Other natural sources may be richer in C but they are either inedible or are not consumed in considerable amounts. For example, both green peppers and parsley are richer than oranges in this vitamin, but they





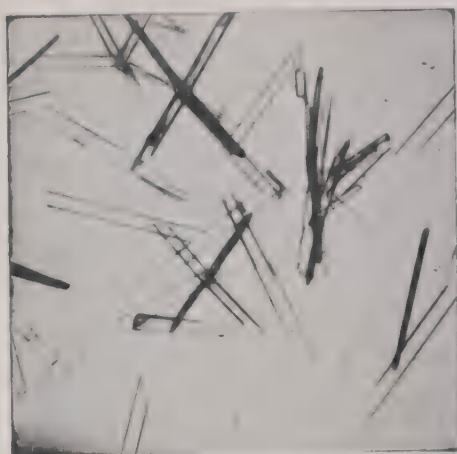
Plate I.—Gingivitis in latent scurvy. (Courtesy of Dr. Tom D. Spies.)





FIG. 38.—Scurvy in an adult. Observe the petechial hemorrhages on both lower extremities and the swollen, brawny right leg and lower thigh. (From Eddy, W. H., and Dallorf, G.: *The Avitaminoses*, ed. 2, Baltimore, 1941, The Williams & Wilkins Co.)

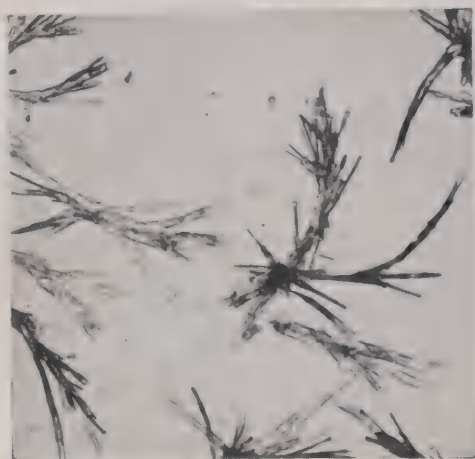




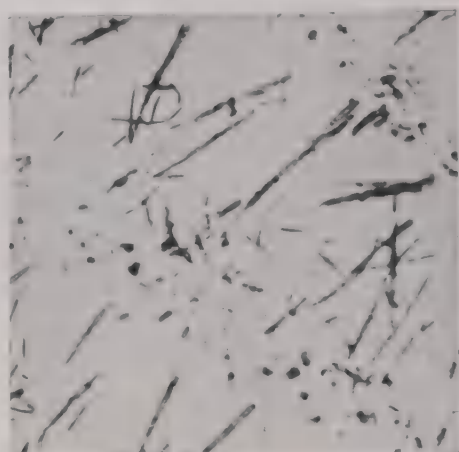
A.



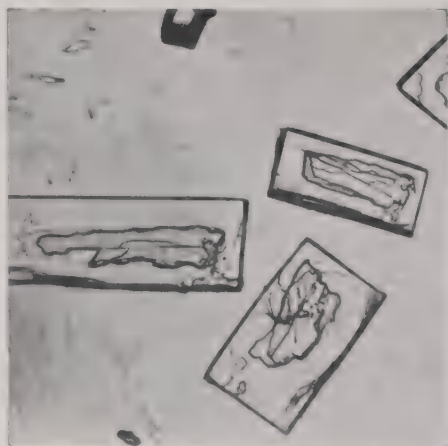
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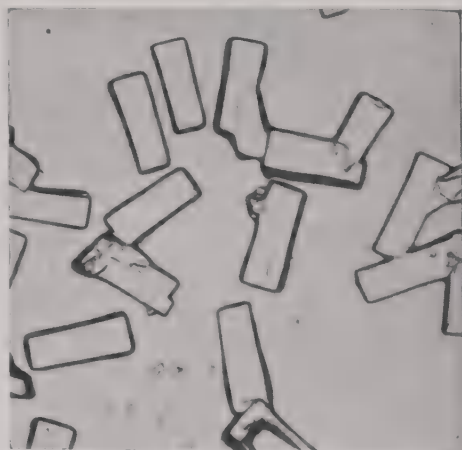
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D.



E.



F.

Fig. 39.—Photomicrographs of vitamin crystals. A, Thiamine; B, riboflavin; C, niacin; D, pantothenic acid; E, pyridoxine; F, ascorbic acid; G, biotin (methyl ester); H, vitamin (alcohol); I, vitamin D<sub>2</sub>; J, alpha-tocopherol palmitate; K, vitamin K; L, vitamin B<sub>12</sub>. (A, C, D, E, F, and L, courtesy Merck and Co., Inc., Rahway, N. J.; G, courtesy Research Laboratories, S.M.A. Corporation, Chagrin Falls, Ohio; and H, I, J, and K, courtesy Distillation Products, Inc., Rochester, N. Y.)

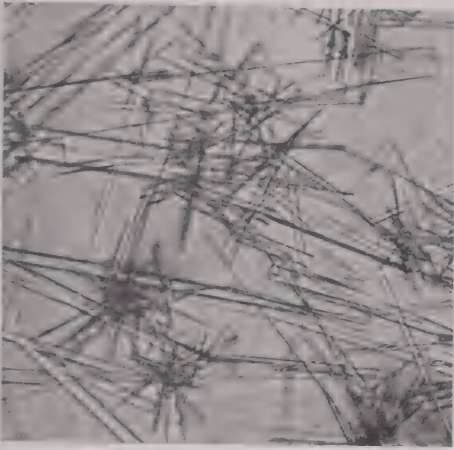
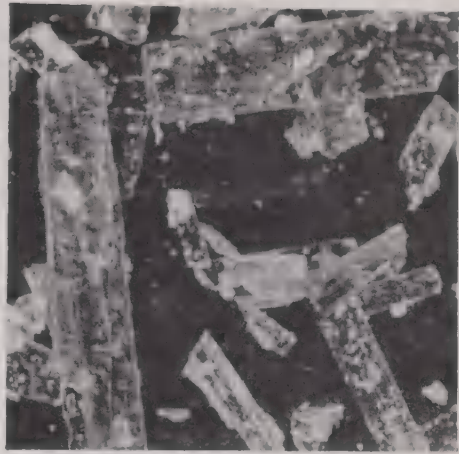
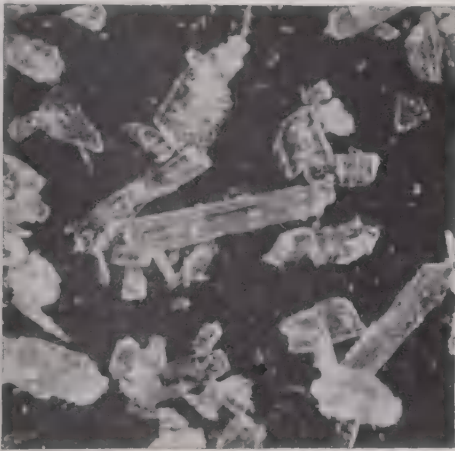
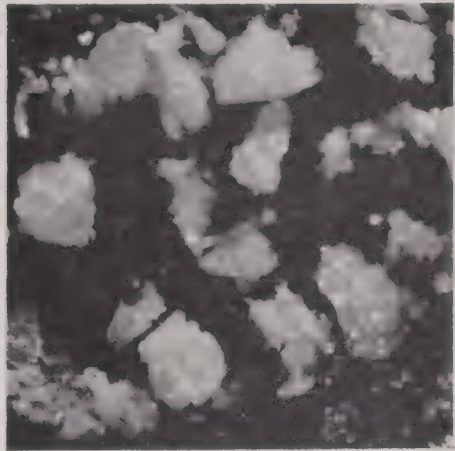
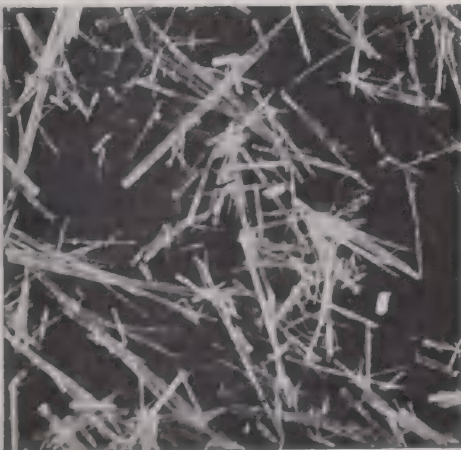
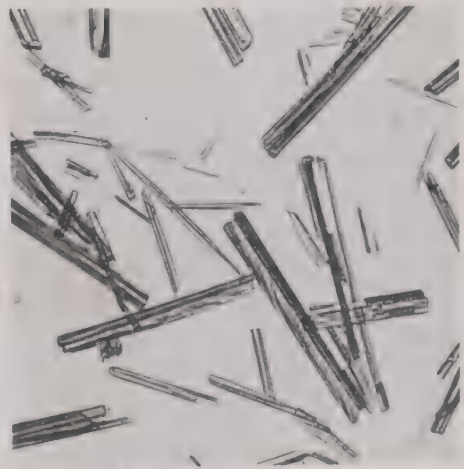
*G.**H.**I.**J.**K.**L.*

Fig. 39 (cont'd).—For legend see opposite page.

not enter into the dietary to any great extent. Spinach and other greens are good sources of it also, but they lose their vitamin C content progressively on storage at room temperature. Citrus fruit juices and tomato juice may be canned with but slight loss of the antiscorbutic factor. However, they should not be permitted to be in contact with air for a long period of time because of loss by oxidation. The practice in hospitals of preparing fruit juices twelve or more hours in advance is to be deplored unless they are kept in closed vessels. Cantaloupes, strawberries, cabbage, and turnips, when raw, are all about equivalent to tomatoes, but the two latter lose some C in cooking. Potatoes, fresh peas, asparagus, and lettuce are good sources also. Ascorbic acid occurs to some extent in animal tissues. In 1928 Szent-Györgi found a "hexuronic acid" with high reducing power in the adrenal cortex and later showed that it had antiscorbutic properties. While this is important when considering the function of the adrenal cortex, it is of little value from the standpoint of food sources of the vitamin because of the almost insignificant quantity of tissue involved. The same is true of corpus luteum, which is said to have a high content of it. Most fresh animal tissues have small amounts of vitamin C. Liver is the best animal source, although fish roe and milt are also reported as being rather rich in ascorbic acid.

Cow's milk contains very small amounts of ascorbic acid which vary with the cow's fodder. In summer, when the cows are in the pasture, their milk is relatively high in ascorbic acid, but in winter, or whenever fresh food is unavailable, the milk has little antiscorbutic value. Human milk has a somewhat higher vitamin C content, but here, too, it is dependent upon the quality of the food. Pasteurizing milk in uncapped bottles has been found to be more destructive of vitamin C than in capped bottles, because the surface of the milk is in contact with the oxidizing influence of the air. It is, accordingly, quite apparent that babies should have supplements of orange juice or tomato juice at least until they receive a varied diet.

**Properties.**—Vitamin C is water soluble and insoluble in fats and oils. It is very sensitive to oxidation, particularly in the presence of copper, but not of aluminum. Therefore, foods prepared in copper vessels or with copper utensils lost this factor quickly. It is also rapidly destroyed by alkalis but is fairly stable in weakly acid solutions. Consequently, baking soda has a harmful effect. Cooking in steam has little destructive action upon the ascorbic acid of foods, if they are neutral or slightly acid, but cooking in open vessels permits oxidations to occur. Drying vegetables usually results in a loss of ascorbic acid but many investigations have been under way in the attempt to provide desiccated foods which shall have all the vitamins, including C, unchanged. Because it is so easily oxidized, it is a strong reducing agent. Freezing has no deleterious effect upon this vitamin.

**Effects of Deficiency.**—The guinea pig is the standard animal for demonstrating vitamin C deficiency and it has been used in the biological assay of foods for this vitamin. At first there is good growth on the vitamin C-free diet, but in about two weeks growth ceases and symptoms begin to appear. The joints become swollen and tender and the animals show signs of pain when



These are pressed. The animals may lie on their sides or assume a peculiar "scurvy" position, lying flat with hindlegs sprawled. They may be excitable at first but soon become very quiet and not easily disturbed. There may also be enlargements of the junctions of the ribs with the cartilages, as well as other bone lesions. Hemorrhages of the gums and loosening or breaking of the teeth may occur. Small amounts of orange juice will change the picture even at a very late stage, and animals may be brought back to an almost normal condition quite rapidly.

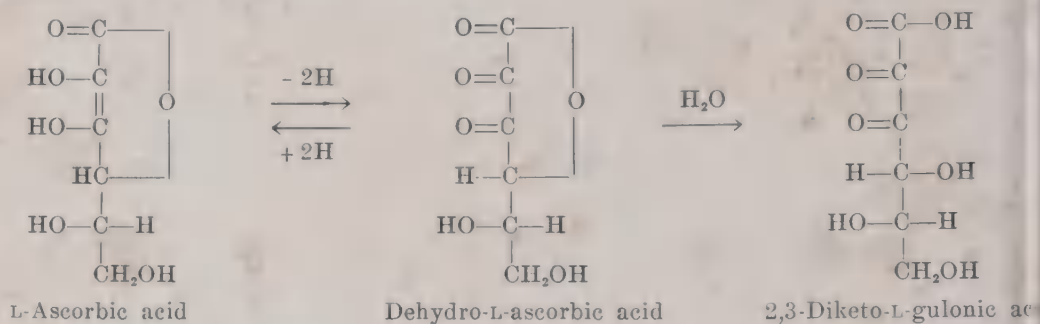
It has been thought that rats and mice require no ascorbic acid in their diets. However, they cannot entirely dispense with it (Kleiner and Tauber), and their ability to get along with exceedingly small amounts is due to the fact that they must synthesize it in their intestines or in their tissues, because it has been shown that the administration of a structural inhibitory analogue will precipitate scurvy (see Chapter 24). Glucoascorbic acid is the substance which can compete with ascorbic acid and cause a vitamin deficiency in mice and also in guinea pigs. (Woolley and Krampitz.) It has an additional  $\text{CHOH}$  in the chain but is otherwise identical with ascorbic acid in structure.

In man an extreme deficiency results in scurvy as already described. But in our ordinary life such marked deficiencies seldom occur. There do occur, however, deficiencies of various grades, due either to a subnormal intake of the vitamin or to an increased requirement. (See Fig. 38.) These deficiencies may result in slow healing of wounds and decreased ability to combat infections and to metabolize amino acids, especially tyrosine, as well as in the scorbutic symptoms already mentioned. It has been shown that vitamin C is a threshold substance; that is, it is not secreted by the kidney until the ascorbic acid level in the blood exceeds a certain value, which in turn depends upon the degree of saturation of the body tissues. It is not stored in the way that vitamins A and D are. As will be seen, there are methods of determining vitamin C chemically which are much quicker than the biological assay. Therefore, the degree of saturation of the tissues with vitamin C may be easily estimated by the aid of such methods. One clinical test is the determination of the concentration of ascorbic acid in blood plasma (Farmer). The normal range is 0.6 to 2.5 mg. per 100 ml., but subnormal values have been found in some cases during pregnancy and lactation and in all cases of scurvy. Some other conditions in which low values have been found are infectious disorders, congestive heart failure, kidney and liver diseases, gastrointestinal disturbances, purpura, endocrine diseases, and malignancies. It cannot be stated that in any of these conditions the lack of vitamin C is a primary causative factor, but it may be significant that the vitamin C blood level is found to be reduced in many pathological states. It is probably a result of increased requirement for the vitamin or a lowered threshold for its excretion, but nevertheless it may contribute to the pathological condition of the patient. After burns, fractures, or extensive surgery, there is also a marked diminution of plasma ascorbic acid.

Another clinical test depends upon the amount of the vitamin excreted in the urine after a test dose of ascorbic acid has been administered. If the tissues are well supplied with the vitamin, a larger amount is eliminated. If they are in need of it, more will be retained. Another interesting test is the ability of the tissues to bleach a dye, sodium 2,6-dichlorobenzenone indophenol.

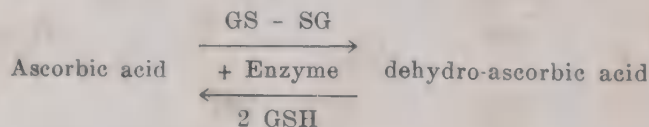
This is the same dye as is used to determine vitamin C quantitatively in blood, urine, and foods. The dye is injected under the skin and forms a blue spot. If no ascorbic acid is present, the color remains, but with increasing concentration of vitamin C in the tissues the spot disappears more and more rapidly. Children on normal diets, except for citrus fruits and tomatoes, quickly develop a lack of vitamin C as shown by this test or by blood analysis, even though no gross clinical symptoms may be present. (Slobody, Benson and Mesterson)

**Structure and Mechanism of Action.**—Ascorbic acid is a hexose derivative. Its formula together with those of two closely related compounds is given below. In fact, it has been shown that glucose, labelled at any one, or all, of its carbons, is converted, in the rat, into correspondingly labelled ascorbic acid. (Horowitz and King.)



As seen from these formulas, L-ascorbic acid and dehydro-L-ascorbic acid are lactone derivatives of the diketogulonic acid. When ascorbic acid is oxidized it loses two hydrogen atoms and becomes the dehydro derivative. The latter may be reduced to the original ascorbic acid form. Both of these are biologically active, but the stereoisomer, D-ascorbic acid, is not. When dehydro-L-ascorbic acid is hydrated it changes to 2,3-diketo-L-gulonic acid which not only is inactive biologically but cannot be converted back to either of the active forms. Since this hydration takes place spontaneously in neutral or alkaline solution it is easily seen that the oxidation of ascorbic acid frequently means its biologic inactivation.

Since L-ascorbic acid is so easily oxidized to its dehydro derivative and vice versa, it is believed that this reaction takes place in the tissues. It may be part of one of the important respiratory enzyme systems. It can be oxidized and reduced by glutathione which may also be a part of the system. Thus:



Besides this action, ascorbic acid has been described as a coenzyme for cathepsin, for liver esterase, and for other enzymes. It also appears to have something to do with the metabolism of tyrosine. (See page 382.) Recent studies indicate that another function is its role in the conversion of folic acid

into a physiologically active form. Whether any of these functions is concerned in the part which ascorbic acid plays in renewing the cement substance to all tissues is not known.

**QUANTITATIVE DETERMINATION.**—The assay of vitamin C in foodstuffs was for a long time possible only by the biological method; i.e., by feeding experiments. Such a method was very time consuming, but now there are chemical procedures which are fairly accurate. These are applicable to blood and urine as well as to foods. All depend upon the reducing action of ascorbic acid. If the *total* vitamin C content (i.e., ascorbic acid plus dehydroascorbic acid) is to be determined, the dehydro form must first be reduced to ascorbic acid. This may be accomplished by bubbling  $H_2S$  through the solution. The most common method of assay is based on the reduction of a dye, 2,6-dichlorophenolindophenol, which changes from blue to colorless as it is reduced and as the vitamin is oxidized. This may be a titration or a colorimetric procedure. Other easily reduced agents such as potassium ferricyanide may also be used.

**Human Requirements.**—For adults the recommended daily allowance is 75 mg. with a minimum set at 30 mg. Infants should have 30 mg. a day, with a 10 mg. minimum, and, as the child gets older, a gradually increasing amount is required until a maximum need is reached at adolescence. At this time, from 80 to 100 mg. are recommended. Even higher amounts are desirable in pregnancy and lactation to provide for the growing fetus and for the secretion of some of the vitamin into the milk.

### The Vitamin B Complex

When the vitamins were first designated "fat-soluble A" and water-soluble B," only one active principle was thought to be present in each. "Water-soluble B" had growth-promoting properties for the rat and cured the polyneuritis which had been produced in pigeons by feeding them polished rice. In man, the disease beriberi was found to result from a deficiency of vitamin B and to yield to treatment with it. Later, pellagra was shown to be due to a deficiency of something present in vitamin B preparations. The designation P-P (pellagra preventive) was at first assigned to it. Additional factors were separated, at first on the basis of varying biological reactions, using different sources and different species; later on the basis of adsorbability by Fuller's earth and other physical or chemical properties. A vitamin B complex factor is, according to Ansbacher's definition, (1) a natural constituent of yeast, liver or cereals, (2) water soluble, (3) a growth-promoting substance for microorganisms, (4) a coenzyme or activator of enzymatic processes, (5) physiologically effective in minute amounts, and (6) a substance, the absence of which in the diet, causes a deficiency disease.

Two systems of nomenclature were used: one giving each factor a different letter, as vitamin B and vitamin G, and the other naming them  $B_1$ ,  $B_2$ ,  $B_3$ , etc. Although the latter system is being used more than the former, there is a growing tendency to abandon both for the definite names of the compounds as soon as they are isolated. Thus, the group includes thiamine ( $B_1$ ), riboflavin



(B<sub>2</sub>), pantothenic acid (B<sub>3</sub>), niacin (B<sub>5</sub>), pyridoxine (B<sub>6</sub>), biotin (B<sub>7</sub>), folic acid (pteroylglutamic acid) (B<sub>9</sub>), p-aminobenzoic acid, and B<sub>12</sub>. In 1926 the group could be separated into two fractions, one stable to heat and unadsorbed on Fuller's earth, the other destroyed by prolonged heating at over 100° C and strongly adsorbed. The latter, the thermolabile group, contains thiamine vitamin B<sub>1</sub>.

### Vitamin B<sub>1</sub>

Vitamin B<sub>1</sub>, or thiamine, has been called the antineuritic or antiberiber factor; in Europe it is designated "aneurin." A marked deficiency of thiamine in the diet results in:

1. Arrested growth of young animals. This is a specific effect on growth not due to the inhibitory influence upon appetite which this vitamin also exerts.

2. Polyneuritis in animals. Birds develop acute polyneuritis after several weeks and are unable to fly, walk, or even to stand. Death occurs unless the vitamin is given. Rats develop, among other symptoms, a bradycardia (slowing of the heart rate). Both the curing of the polyneuritis of pigeons and of the bradycardia of rats have been used in methods of biological assay.

3. Beriberi in man. This disease is common in the Orient but also occurs in other parts of the world. In the adult it is characterized by polyneuritis with muscular atrophy, cardiovascular changes, and edema (Plate II). At first there is weakness and fatigue, followed by headache, insomnia, and dizziness, loss of appetite, and other gastrointestinal symptoms, and tachycardia. Later the major symptoms may follow chiefly one of the following patterns: (a) nervous symptoms (*dry beriberi*); (b) symptoms associated with edema and serous effusions (*wet beriberi*); (c) symptoms of heart involvement (*acute pernicious beriberi*). Often the symptoms will be characteristic of more than one of these three classes and are called *mixed beriberi*. Although beriberi is a thiamine deficiency disease, it is almost always accompanied by deficits of other vitamins. This is true of all vitamin B complex deficiency conditions in man, and perhaps of others as well.

Beriberi in infants results when their diet is restricted to the milk of mother suffering from beriberi and undoubtedly is due to a lack of thiamine in the milk. It occurs suddenly. The symptoms include rigidity of the body, constipation, diminished flow of urine (oliguria), a peculiar whining, weakness, edema, enlargement of the heart, cyanosis, and a rapid, irregular pulse.

**Occurrence.**—The vitamin is present in many plant and animal foods. Whole grains, legumes, beef, pork, liver, nuts, and yeast are the best sources, while fair sources are eggs, fish, and many vegetables. However, although widely distributed, many foods have such small amounts present that partial thiamine deficiencies can easily occur. The milling of wheat flour has lowered its thiamine content more than 80 per cent; as a consequence, enrichment of white flour or of white bread with thiamine is widely practiced. Furthermore, because of its solubility in water, much B<sub>1</sub> may be lost if the water in which foods are cooked is discarded. The desirability of utilizing these "cook waters" for soups, gravies, and sauces is evident.



Plate II.—Pitting edema of the leg in thiamine deficiency. (Courtesy of Dr. Tom D. Spies.)





Foods may be classed in general into three groups in regard to their thiamine content:

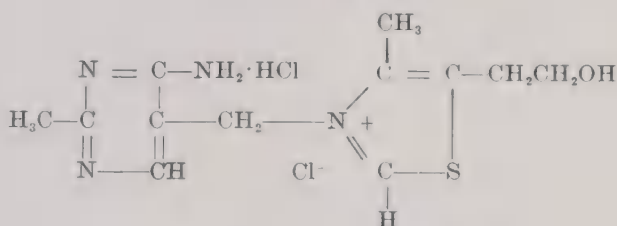
I. The foods highest in thiamine are whole cereals, lean pork, heart, and kidney, although none of these is rich to the same degree as are some foods in vitamins A, C, and D.

II. Foods high in thiamine consumed in relatively small amounts, such as yeast; and foods low in thiamine but consumed in relatively large amounts: meats other than those mentioned in Group I, milk, fresh fruits, and vegetables. The livers and roe of fish are reasonably good sources of thiamine, as well as of the other vitamins of the B complex.

III. Foods quite deficient in thiamine: white flour (not enriched), polished rice, white breakfast cereals, spaghetti, macaroni, refined cane sugar, and molasses.

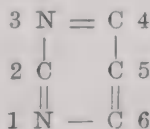
**Properties.**—Thiamine is a white, crystalline compound, readily soluble in water, slightly soluble in ethyl alcohol, but insoluble in ether and chloroform. It has the odor and flavor characteristic of yeast. The aqueous solution has an acid reaction and is optically inactive. In the dry condition it is relatively stable to heat up to 100° C. but is slowly destroyed by moist heat. Acid retards and alkali hastens this destructive action. In cooking, thiamine is not destroyed to any great extent if the temperature is not much above 100° C., if the reaction is not alkaline, and if the heating is not continued for too long a time.

**Structure.**—The structure of thiamine has been determined to be:

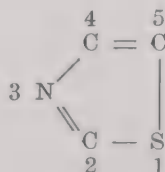


Thiamine chloride hydrochloride

It has been synthesized and the synthetic vitamin is frequently used in medicine. It occurs in nature either as the free vitamin or as the pyrophosphate. In studying the structure of thiamine it should be observed that it contains a pyrimidine nucleus and a thiazole nucleus:



Pyrimidine



Thiazole

No other natural compound contains thiazole, although various biological substances contain sulfur. The pyrimidine is also unique in that it is the only natural pyrimidine having an alkyl group in position 2. It is interesting to note that plants can use a mixture of pyrimidine and thiazole compounds in place

of thiamine itself, whereas animals require the complete vitamin. An apparent exception to this latter statement is the discovery that polyneuritic pigeons may be cured by being fed very large doses of a mixture of these intermediates. Perhaps a preliminary synthesis is brought about by microorganisms in the intestinal tract.

**Mechanism of Action.**—Thiamine is involved in the intermediary metabolism of carbohydrates in all the cells of the body. Several oxidative phenomena have been shown to depend upon it, and probably they are related to each other. Brain tissue from pigeons having severe thiamine deficiency takes up oxygen at a lower rate than normally. This rate can be increased by adding thiamine to the tissue. Such tissue is also found to have an excess of lactate and pyruvate, and it has been suggested that the neuritic symptoms may be a result of excess of pyruvate. The vitamin, linked to phosphoric acid, becomes thiamine pyrophosphate. This is a coenzyme, "cocarboxylase," and is essential to a number of enzyme reactions including the one in which pyruvic acid is decarboxylated (see Chapter 16). Usually the content of cocarboxylase in blood parallels that of thiamine. However, in diabetes mellitus there is a high thiamine content with a low cocarboxylase. The suggestion is that insulin is necessary for the normal processes of thiamine phosphorylation; and the lack of this coenzyme results in a failure of the oxidative reactions in diabetes (Goodhart and Sinclair; Siliprandi and Siliprandi.)

**Human Requirements.**—Thiamine is not stored in the tissues to any great extent and loss of this water-soluble substance continually occurs by way of the urine. Apparently the thiamine content of the tissues varies somewhat with the amount in the food, and, consequently, following a period of overabundance, a temporary reserve will be built up sufficient to take care of the individual for a few weeks. Ordinarily, however, any loss must be made good soon after it occurs. There are various factors which influence the requirement and all may be related to the amount of carbohydrate metabolized: (a) age—children require more per kilogram of body weight than adults; (b) activity—thiamine needs vary with caloric requirements; (c) pregnancy and lactation—here again greater amounts must be provided for the fetus or the suckling infant; (d) diet—high carbohydrate increases the need for thiamine. Substitution of fat for carbohydrate decreases the thiamine requirement, while protein seems to have no specific effect. The recommended allowances are about 1.5 or 2.5 mg. per day (0.5 to 1.0 mg. per 1,000 calories) (see Table XXIX, page 326).

In view of its limited distribution in foods, thiamine is almost the only vitamin which may be lacking even in a fairly good diet. In patients on a restricted diet, or with a diuresis leading to a rapid loss, the deficiency may be very real. Administration of thiamine under these circumstances is widely practiced.

If raw clams, or certain other raw seafood, are included in the diet, a thiaminase present may destroy enough thiamine to produce a deficiency. (Melnick.) The enzyme action is a cleavage between the pyrimidine and thiazole rings. Horses and cattle which consume large amounts of fern sometimes become ill of "fern poisoning." This has been shown to be another type of thiamine antagonism, not enzymic in nature, since heated ferns are ju-

s effective. (Weswig.) Several possible explanations have been offered, among them the idea that an inhibitory structural analogue, such as pyrithiamine, may be present in this plant. (See Chapter 24.)

**Other Clinical Applications.**—Clinically the administration of thiamine has met with considerable success in a number of conditions besides frank beriberi. Indeed, beriberi is very infrequently seen except in the Orient. As might be expected, other types of neuritis have been treated with thiamine on general principles." Alcoholic neuritis and pregnancy neuritis seem to be due to a lack of this vitamin, and definite improvement is usually seen upon treatment with thiamine alone, or, preferably together with other constituents of the vitamin B complex. If the neuritis is not associated with a thiamine deficiency, the administration of thiamine does not help, nor does it if permanent destruction of nervous tissue has taken place.

In various forms of nutritional deficiency there are symptoms of cardiovascular disturbance. Weiss and Wilkins showed that B<sub>1</sub> administration usually ameliorated these symptoms even though the patient was suffering from a lack of several vitamins. Gastrointestinal disorders, also, have been ascribed to thiamine deficiency. Lack of appetite and loss of muscular tone of the stomach and intestine were first shown in animals. It is more difficult to correlate a vitamin deficiency with gastrointestinal symptoms in man because of the great number of other factors which enter into the picture. However, several investigators believe that a certain group of symptoms is frequently caused by lack of thiamine. These include loss of appetite, low gastric HCl, atony of the stomach and intestines, constipation, and a marked tendency toward the development of intestinal inflammatory processes. Treatment of gastrointestinal conditions with thiamine (and other vitamins) may require parenteral administration since the intestinal disturbance may operate to prevent absorption of the vitamin.

### The Heat-Stable B Vitamins

The heat-stable fraction of the "original vitamin B" has been found to comprise a number of different vitamins, frequently called the B<sub>2</sub> complex. These are riboflavin, niacin, pyridoxine, pantothenic acid, and biotin. Choline, aminobenzoic acid, and inositol are also of nutritional importance but are not considered to be true vitamins. They will, however, be discussed in this chapter.

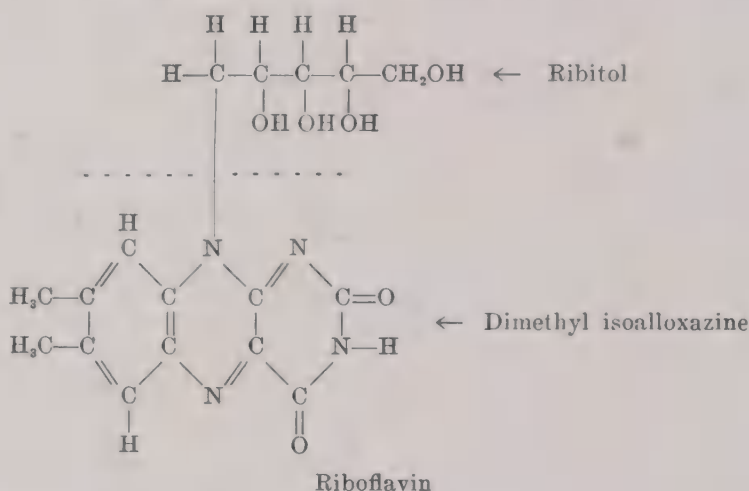
### Riboflavin

No recognized disease is associated with an exclusive deficiency of riboflavin, but in pellagra, which is due to a lack of niacin, there is usually also a lack of riboflavin. In rats a riboflavin-free diet causes, besides a cessation of growth, vascularization of the cornea, frequently a loss of hair, and scaliness of the skin (with pediculosis); later, cataracts may develop. Dogs also develop cataracts if deprived of riboflavin. In the human being it is doubtful whether cataract is a result of this deficiency. In man there is cheilitis, a condition which is characterized by inflammation of the lips, fissures at the corners of the mouth, dryness, greasiness, and fissures in the folds of the ears and nose (Plate III).



Some initial trauma or infection is likely to be followed by a skin lesion if riboflavin deficiency is present. (Sebrell and Butler; Horwitt.) There may be ocular disturbances such as inflammation of the cornea, bloodshot eyes, photophobia, dimness of vision, and itching, burning, and dryness of the eyes with redness of the conjunctiva. The increase in the blood supply to the eyes may be an attempt to furnish oxygen by means of oxyhemoglobin to tissues which ordinarily depend more upon the respiratory functions of the vitamin than upon the respiratory pigment of the blood.

**Properties.**—Riboflavin is an orange-yellow crystalline compound. It is water soluble and heat stable, especially in acid solution, but very easily decomposed by exposure to light. Its water solution exhibits a yellow-green fluorescence. It is a pigment consisting of dimethylisoalloxazine attached to ribitol. In nature it may occur as the free pigment, as riboflavin phosphate, or as a constituent of flavoproteins. Its structural formula is:



**Distribution.**—Riboflavin occurs widely in nature. Milk is an important source of it. Lactoflavin, one of the pigments of milk, is identical with riboflavin. Other excellent sources are meats, especially liver and kidney, fish, and eggs. Leafy vegetables are richer in riboflavin than they are in thiamin. Fruits and most root vegetables contain moderate quantities. Whole grain cereals, and milled flour are not good sources.

**Mechanism of Action.**—The flavoproteins, that is, combinations of riboflavin with proteins, are enzymes which function in tissue respiration. These are termed “yellow enzymes” because they all contain the orange-yellow riboflavin or some similar substance and include the “yellow enzyme” of Warburg, L- and D-amino acid oxidases, diaphorase, cytochrome c reductase, liver aldehyde oxidase, and xanthine oxidase. They are all dehydrogenases and can alternately be oxidized and reduced. All of them have extremely important intracellular activities and emphasize the necessity of adequate riboflavin supplies in the diet.

**Requirements.**—The recommended daily allowances of riboflavin are about 1 or 2 mg. for children and 2 or 3 mg. for adults. A well-diversified dietary will furnish these amounts, but it has been pointed out that appetite alone may lead the individual to select food with a distinct deficiency in riboflavin.



Plate III.—Cheilitis and photophobia following vitamin B complex deficiency. (Courtesy of Dr. Tom D. Spies.)



Plate IV.—Early glossitis of vitamin B complex deficiency. (Courtesy of Dr. Tom D. Spies.)





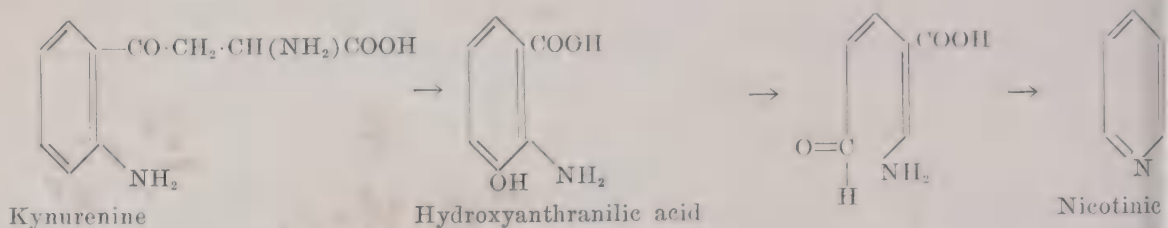
**Excretion.**—Riboflavin is excreted predominantly in the feces and to a lesser extent in the urine. During riboflavin avitaminoses this small urinary fraction diminishes greatly. It is excreted mostly in the free form but in varying amounts as the phosphoric acid ester. Another flavin, uroflavin, is also found in the urine. It is similar chemically to riboflavin but appears to be more soluble in water and to contain more oxygen. It is probably derived from riboflavin.

### Niacin (Nicotinic Acid)

Pellagra is a disease which was long prevalent in southern Europe and in southern United States. Most cases occurred in the low income groups, where diet was restricted to a few cheap foodstuffs. Concurrent with monotony of diet was the crowded unsanitary housing of the poor. This coincidence of circumstances led the early investigators to two hypotheses: the disease must be due either to a nutritional defect or to an infection. The latter hypothesis had many supporters, and it was with great difficulty that the nutritional nature of the disease was established. We owe this chiefly to Goldberger and his colleagues in the United States Public Health Service. They conducted a long series of investigations, the most interesting of which was the prison farm experiment. Twelve convicts were promised pardons if they would agree to subsist on a diet of cornmeal, cornstarch, sweet potatoes, rice, syrup, and pork fat for a year. This was the diet which Goldberger knew was typical of that consumed by pellagrous families. One of the subjects found the diet so much worse than the regular prison farm fare that he refused to continue. The others kept on with the diet, under the same sanitary conditions as the other prisoners on regular prison fare. Before the year was up more than half of the subjects showed symptoms of pellagra, while no such symptoms appeared among the prisoners on the usual diet. At first it was thought that an amino acid deficiency was responsible. Later it was considered to be purely a vitamin problem, and in 1926 Goldberger discovered that yeast, heated to destroy its thiamine, still had curative action on pellagra. This was provisionally called the pellagra-preventive (P-P) factor. Elvehjem and his co-workers proved that nicotinic acid (niacin) and its amide were capable of curing "blacktongue," a deficiency disease of dogs, and soon it was shown to be identical with the pellagra-preventive factor. However, investigators were confronted with a number of facts about pellagra which could not be explained by a purely vitamin theory. One of these was that for the cure of pellagra not only niacin was needed, but also adequate amounts of good quality protein foods, such as milk, which is low in niacin. Another fact was that a diet composed largely of corn led to the development of pellagra, even though an apparently sufficient amount of niacin was present. Animal experiments substantiated this and later led to the observation that addition of casein to the diet has an effect which counteracts that of the corn. Soon the amino acid tryptophan was found to be the factor, lacking in corn proteins, but present in casein, which simulated niacin. (Krehl.) The explanation of this niacin-tryptophan relationship is as follows:

Tryptophan can be transformed into niacin by the body tissues and thus contributes to the body's supply of the vitamin. This could occur in the following manner. Tryptophan is normally converted to kynurenine (see

page 383); this may be oxidized in the liver and kidney to hydroxyanthranilic acid, which has been shown to substitute in animals for niacin (Mitchell Priest).

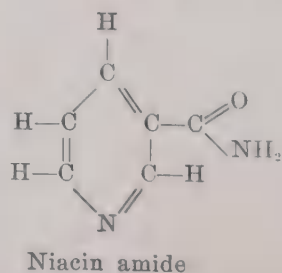
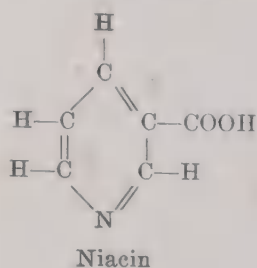


For this transformation the presence of pyridoxine seems to be necessary (Hurt; Bonner and Yanofsky).

In pellagra there occur patches of dermatitis, soreness and inflammation of the tongue and mouth; alimentary disorders including achlorhydria and diarrhea, and pigmentation and thickening of the skin (Plates IV and V). There is usually a rash which appears symmetrically on the sides of the body and the backs of the hands and arms. The pigmentation of the skin may persist for years after the dermatitis has healed. Nervous disorders and mental disturbances occur, particularly in the later stages. Some of the mental symptoms seen in chronic alcoholism have been ascribed to a niacin avitaminosis, at least in part, since the diet of many alcoholics is deficient in all of the B vitamins. While these are the typical pellagrous symptoms and the administration of niacin relieves them, as a rule other symptoms may accompany them; also, as a rule, the administration of niacin does not entirely cure the patient. The reason is that, together with the deficiency of niacin, there is usually a deficiency of riboflavin and thiamine as well. Often other food factors are missing so that other vitamins may have to be administered. A well-rounded and complete diet must be insisted on in order to prevent the recurrence of the symptom complex.

**Properties.**—Although niacin was discovered as a vitamin comparatively recently, the compound was known long before the “vitamin era.” Since it could be produced by the oxidation of nicotine, it has been known as nicotinic acid. This name was considered misleading by some and therefore the terms niacin and niacin amide, respectively, were coined for the acid and its amide, although nicotinic acid and nicotinamide are again being preferred. When pure, it occurs as white, needlelike crystals. It is water soluble and stable in air, and also to heat. There is little loss in cooking unless the “cook water” is discarded.

**Structure.**—The structural formulas of the acid and the acid amide are





A.



B.

Plate V.—Results of severe niacin deficiency. A, Lesions of the hands in pellagra; B, same patient after treatment with niacin amide. (Courtesy of Dr. Tom D. Spies.)





**Occurrence.**—Niacin is found in largest amounts in meats, especially liver. Fish and eggs are also good sources, as are some cereals and vegetables, notably whole wheat and unpolished rice, and peanuts. However, a number of our staple vegetable articles of diet are not particularly rich in niacin and therefore vegetarian diets may be lacking in this vitamin. While whole wheat is an excellent source of niacin, most of this vitamin, like thiamine, is lost in the milling process. It is now one of the substances used to "enrich" white flour or white bread.

**Effects of Deficiency in Animals.**—The dog, pig, and monkey are the only experimental animals which exhibit symptoms as a result of niacin deficiency. Chittenden and Underhill in 1917 described a condition in dogs known as "blacktongue." This is characterized by a sudden refusal to eat the deficient diet, apathy, and lesions in the mouth. The inner surface of the lips and cheeks become covered with pustules and the mucous lining comes away in shreds. Intense salivation and bloody diarrhea are additional symptoms, and there may be pustules on the thorax and upper abdomen. In monkeys the chief symptoms are tender bleeding gums leading to ulceration and necrosis of the gum tissues. Vincent's infection is likely to set in and if niacin is not provided, monkeys die usually in from two to six weeks.

**Mechanism of Action.**—Niacin has been shown to be a part of two very important coenzymes: coenzyme I and coenzyme II. These are nucleotides, the structural formulas of which are shown on page 347. They are members of coenzyme systems which are concerned in cellular respiration and in the breakdown of sugar. As will be seen, niacin and riboflavin are intimately associated in some of these reactions.

**Human Requirements.**—The recommended allowance for children is from 5 to 20 mg. per day and for adults, from 15 to 20 mg. Active muscular work, pregnancy, and lactation increase the requirements to some extent. Large doses of niacin or niacin amide are not toxic. Up to 2 Gm. per kilogram of body weight have been given to human beings without any toxic effects resulting.

### Pyridoxine

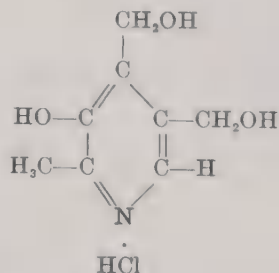
Pyridoxine (vitamin B<sub>6</sub>) is another pyridine derivative which belongs to the heat-stable B complex. It has been called the "antiacrodynia factor," acrodynia meaning affection of the extremities. In pyridoxine deficiency in rats there is a swelling of the ears and dermatitis of the paws and of the nasal region, followed by incrustation (Fig. 40). Although it has been called rat pellagra, it has no relation to human pellagra; in fact, no symptoms have yet been observed in the human being as a result of pyridoxine deficiency, but this does not necessarily mean that man does not require this vitamin. Other species which do show symptoms when deprived of pyridoxine are the chick, the dog, the pig, and the rhesus monkey. These symptoms include epileptiform seizures in rats, dogs, and pigs and a characteristic anemia in dogs. In the monkey arteriosclerosis develops, but whether this has any relation to the disease in human beings is not known. (Rinehart and Greenberg.) Pyridoxine is water

and alcohol soluble and is slightly soluble in fat solvents. It is sensitive to light, ultraviolet irradiation, and alkali. It is heat stable. The foods which are richest in pyridoxine are egg yolk, meat, fish, and milk among animal sources, and whole grains, cabbage, and legumes.

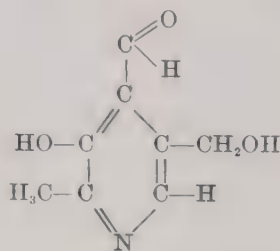


Fig. 40.—Pyridoxine (vitamin B<sub>6</sub>) deficiency in a rat, characterized by dermatitis of the extremities, beginning with swelling of the ears, nasal region, and paws, and followed by crust formation on these areas. (Courtesy Research Laboratories, S.M.A. Corporation, Chagrin Falls, Ohio.)

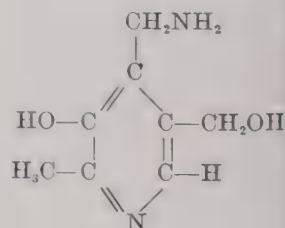
Pyridoxine hydrochloride is a white crystalline substance. Its formula and those of two derivatives, which are quite important, are:



Pyridoxine hydrochloride



Pyridoxal



Pyridoxamine

As to the more specific functions of pyridoxine, they seem to be related to the utilization of the unsaturated fatty acids and the amino acids. Pyridoxine is thought to be essential to the metabolism of unsaturated fatty acids and to the



biological conversion of protein to fat. The change from pyridoxal to pyridoxamine and vice versa may be required in transamination reactions (Lichstein). (See page 366.) Pyridoxal phosphate is the coenzyme for the decarboxylases which act upon a number of amino acids and for two enzyme systems involved in the metabolism of sulfur-containing amino acids (see page 386) (Gunsalus and Bellamy). It also appears that pyridoxine is essential for the normal metabolism of tryptophan. (Lepkovsky.) In its absence, large amounts of xanthurenic acid, a product of the incomplete metabolism of this amino acid, are excreted in the urine and for the conversion of tryptophan to niacin, pyridoxine is needed.

Although pyridoxine is undoubtedly required by man, it has been difficult to provoke symptoms of pyridoxine deficiency. Long periods of deprivation are required before any effects are noted. These include a fall in the hemoglobin and alteration of the white blood cell relationships, depression, and mental confusion. By the use of a structural antagonist, however (see Chapter 24), skin lesions can be produced in a shorter time. These resemble the ones which occur in riboflavin and nicotinic acid deficiencies.

### Pantothenic Acid

Pantothenic acid was at first called the "filtrate factor" and was given its present name by its discoverer, R. J. Williams. It was so called because of its widespread occurrence. A deficiency causes dermatitis in the chick and graying of the hair in black rats; this, however, is not the only "antigray hair factor." (Fig. 41.) In the rat there are seen also dermatitis and inflammation of the nasal mucosa, "spectacled eye condition" (which is more characteristic of biotin deficiency). There are also hemorrhages in certain organs, especially the adrenal cortex.



Fig. 41.—Pantothenic acid deficiency in a rat. These rats were litter mates, both originally black. Their diet, after weaning, contained no pantothenic acid, but the one on the left received 100 gammas of this vitamin daily. After three weeks on this diet the animal on the right showed evidences of graying which gradually became more pronounced. Other deficiency symptoms included scaly dermatitis, inflammation of the nasal mucosa, and hemorrhages in various organs, particularly in the adrenal cortex. (Courtesy Research Laboratories, S.M.A. Corporation, Chagrin Falls, Ohio.)

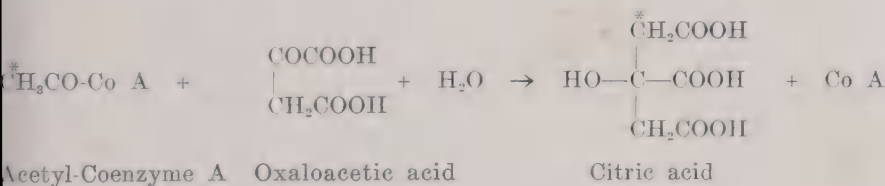
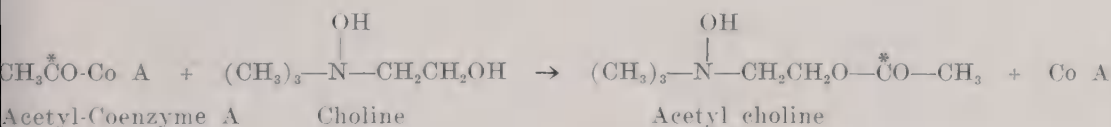


out by a number of investigators (Novelli). It is an atypical dinucleotide (see page 398), the usual mononucleotide being replaced by "phosphopantetheine." "Pantetheine" is pantothenic acid, joined to  $\beta$ -mercaptoethylamine through a peptide linkage. At the other end, pantothenic acid is joined by a pyrophosphate bridge to an adenylic acid group. This adenylic acid (see page 404) consists, of course, of adenine, ribose, and phosphoric acid, but the phosphoric acid is linked on to the number 3 carbon of the ribose.

In order to effect an acetylation, coenzyme A must be present in the form of acetyl-coenzyme A. This is what was known as "active acetate" until its identity was established. It can arise as follows: Coenzyme A, in the presence of adenosine triphosphate (ATP, see page 422) and acetate, and a suitable enzyme, is converted into acetyl-coenzyme A. The over-all reaction, which was worked out in Lipmann's laboratory, may be shown in three steps. (Jones.)

- (a)  $\text{ATP} + \text{enzyme} \rightleftharpoons \text{Adenylic acid-enzyme} + \text{pyrophosphate}$   
 (b)  $\text{Adenylic acid-enzyme} + \text{Co A} \rightleftharpoons \text{Co A-enzyme} + \text{adenylic acid}$   
 (c)  $\text{Co A-enzyme} + \text{acetate} \rightleftharpoons \text{Acetyl-Co A} + \text{enzyme}$

The acetyl group may be transferred to an acetyl acceptor in the presence of a suitable enzyme. This may occur in two ways. The acetyl radical may be attached to the accepting group either at the carbonyl or at the methyl end. For example:



The asterisk indicates, in each instance, the carbon attached. Other functions of coenzyme A will be discussed in subsequent chapters.

Reactions of this nature have been shown to result in the synthesis of acetoacetic acid from two molecules of acetic acid (Soodak and Lipmann), and of citric acid from the acetylation of oxaloacetic acid (Stern and Ochoa). The products of these reactions enter into carbohydrate and fat metabolism, as will be brought out later. Furthermore, the acetylation of sulfanilamide and related compounds results in the formation of a peptide linkage (page 647). Thus, pantothenic acid may take part in protein synthesis.

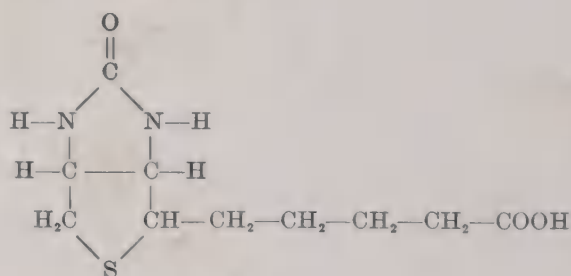
Another interesting possibility is that the physiology of skin and hair pigmentation is in some way dependent upon both the adrenal cortex and pantothenic acid. This is indicated by the fact, shown by Ralli and Graef, that graying of the hair (achromotrichia), which develops in rats as a result of pantothenic acid deficiency, may be reversed by the removal of the adrenal glands.



## Biotin

Biotin is a food factor which for a long time was known to be necessary for the development of microorganisms. Biotin deficiency cannot be readily induced in animals by feeding biotin-free diets, nor is such a deficiency seen in man. It is produced by feeding large quantities of egg white. If rats are fed such a diet, they develop a characteristic group of symptoms including an extensive dermatitis, with "spectacled eye" and involvement of the nervous system (Fig. 42). This may be prevented by addition of yeast or other food rich in biotin. The explanation for these facts is that egg white contains a protein called "avidin," which is responsible for the egg white injury. Avidin combines with biotin in a firm linkage to form a compound which the body cannot absorb, and which is therefore excreted. Thus an induced biotin deficiency, or egg white injury, results. Apparently under ordinary conditions the intestinal bacteria synthesize sufficient biotin for the needs of the animal.

**Properties and Structure.**—Biotin crystallizes in long needles and is soluble in water and ethyl alcohol but is insoluble in ether and chloroform. It is heat stable. The structure of this compound has been worked out by Du Vigneaud and his colleagues. It is as follows:



Biotin

Biotin is said to occur in foods both free and combined. The combined form is easily liberated by the action of proteolytic enzymes, and therefore the linkage is believed to be of a peptide nature. (Bowden and Peterson.)

**Occurrence.**—Biotin is widely distributed in both the animal and vegetable kingdoms. Excellent food sources are liver, kidney, milk, and molasses. There is some evidence that "biotin vitamins," which do not combine with avidin, also occur. It also appears that a large part of the biotin absorbed is synthesized by the intestinal flora. Therefore it is impossible to fix a definite requirement for man, and a deficiency is hardly likely to occur.

**Function.**—The role of biotin in physiology is not clear. It may be involved in fat metabolism since feeding it to rats on a low protein and low vitamin B complex diet produces an increased deposition of fat and cholesterol in the liver. Other possibilities include an influence upon decarboxylation and carbon dioxide fixation (Lardy) and upon the deamination of certain amino acids. (Lichstein.)

## Para-Aminobenzoic Acid

In 1941 it was found that failure of lactation occurred in rats whose diets contained all the known B vitamins, including thiamine, riboflavin, niacin

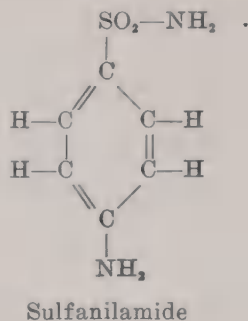
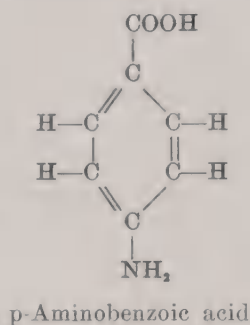
pyridoxine, pantothenic acid, and choline (Sure). It could be cured by yeast and other sources of the B complex. Graying of the hair of black rats also resulted when animals were fed on such a diet (Ansbacher). Both have now been related to para-aminobenzoic acid which had previously been shown to be necessary for growth in the rat and chick and also for bacterial multiplication. As mentioned before, pantothenic acid has also been described as an antigray hair factor, and it has been claimed that both biotin and folic acid have similar properties. It is quite possible that graying of the hair in man may sometimes be the result of a nutritional deficiency. For instance, Greeley, the Arctic explorer, became gray after a nine-month period of undernutrition. After he had eaten a normal diet for a while his hair darkened perceptibly. Since the experimental field is still in confusion, it is not surprising that results are not clear cut in human beings, but neither para-aminobenzoic acid nor pantothenic acid (see Fig. 41) seems to be the responsible factor in man.



Fig. 42.—Biotin deficiency in a rat. This animal had been on a diet containing 35 percent uncooked dried egg white as the sole source of protein. Alcoholic extract of yeast supplied vitamin B complex. Although not biotin-free, the uncooked egg white combines with the biotin, making this unavailable to the animal. This deficiency is characterized by swelling and redness of the lips, denuded areas, and brown scaldiness of the skin with extensive dermatitis. Later the eyes become gummed shut, the edema of the paws increases, and nerve involvement is shown by progressive spasticity. In advanced cases the rat exhibits the so-called "kangaroo posture" as seen here. (Courtesy Research Laboratories, S.M.A. Corporation, Chagrin Falls, Ohio.)

It has been known that para-aminobenzoic acid (PABA) blocks the bacteriostatic effect of sulfanilamide in vitro. The explanation offered for this is that there is competition between the two substances in some vitally important enzyme system. Para-aminobenzoic acid is synthesized by the bacteria and is an essential metabolite for the bacterial cell. It takes part in some enzyme reaction necessary for the life of that cell, possibly in a phenolase system. Because of its structural resemblance to para-aminobenzoic acid, sulfanilamide (para-aminobenzenesulfonamide) takes its place in the enzyme system but does not permit

the vital reaction to proceed normally. Other sulfa drugs have similar action, although they differ quantitatively. It is interesting to note that the bacteriostatic potency of each sulfa drug is directly proportional to its ability to counteract the antibacteriostatic action of p-aminobenzoic acid.



It is interesting to note that PABA forms part of the folic acid molecule (see page 301). This may be the point of attack by the sulfa drugs. Some investigators have questioned whether PABA of itself has catalytic actions and hence have not accepted it as a true vitamin. In view of this antagonism between the vitamin and the sulfonamide drugs, the continuous ingestion of extremely large doses of PABA is to be avoided. In itself it is relatively non-toxic, but the presence of a high PABA level in blood and tissues might render sulfonamide therapy of little value.

**Properties and Occurrence.**—Para-aminobenzoic acid is a crystalline white compound, slightly soluble in cold water, but quite soluble in hot water and in alcohol. It is widely distributed in nature but is more concentrated in yeast, rice bran, and whole wheat.

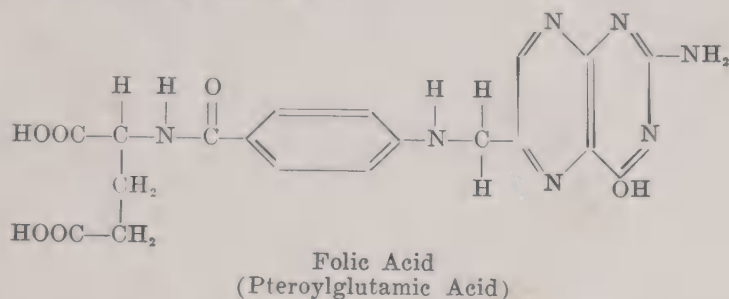
### Pteroylglutamic Acid ("Folic Acid")

The discovery of the folic acid group of vitamins is the result of many different investigations. The research proceeded along two chief lines, however. Certain substances were found to be essential to the growth of microorganisms, particularly *Lactobacillus casei* and *Streptococcus lactis* R. Other investigations dealt with factors found to be necessary in the nutrition of chicks, guinea pigs, monkeys, and other species of higher animals. Since these substances were not the same as the known vitamins they were given new names as their functions became apparent. It was not until 1941 that it was evident that the chick vitamin and the bacterial growth factor were probably a single substance. (Hutchings.)

The vitamin to which all of these vitamins is related is pteroylglutamic acid (PGA). This is the *Lactobacillus casei* factor, isolated from liver, first shown to be a factor necessary for the growth of that organism. A number of other compounds, isolated from other sources, and having similar or even different biological properties, have been found to be closely resembling substances. In other words, liver *Lactobacillus casei* factor, fermentation *Lactobacillus casei* factor, folic acid, vitamin B<sub>c</sub> (Hogan), vitamin M (Day), factors R, S, and U, and yeast norite eluate factor are all vitamins of the same group. All are related to the

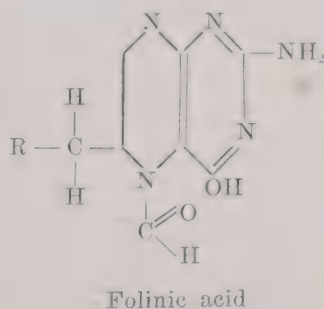


compound now called pteroylglutamic acid. The structure and synthesis of this compound was worked out by the groups at the Lederle and the American Cyanamid Laboratories. (Angier.) It is composed of three main parts: (1) a two-ringed nitrogenous compound called a "pteridine," a yellow pigment first isolated from butterflies' wings, (2) p-aminobenzoic acid, and (3) glutamic acid. It has the following structure:



The related vitamins differ in two respects. The first difference is in the number of glutamic acid groups present, the additional glutamic acid molecules being conjugated in peptide linkages. The commonly occurring ones are the monoglutamate (PGA), the triglutamate (fermentation factor), and the heptaglutamate. The conjugates, i.e., those compounds having more than one glutamic acid in the molecule, are ineffective for some species which do not possess the "conjugase" necessary to release the free vitamin. Normally a conjugase is present in human blood.

The second difference is in the structure of one of the rings, which occurs when PGA is converted to folinic acid, the "citrovorum factor" (CF). This is so called because it supports the growth of *Leuconostoc citrovorum*, which the other members of this group are unable to do. More important, however, is the fact that PGA can be converted to the citrovorum factor, which is perhaps a thousand times more active biologically. Vitamin B<sub>12</sub> (and also ascorbic acid) is involved in this conversion of PGA to CF either directly or indirectly. The citrovorum factor, folinic acid, or, to a much less degree, PGA is concerned in the production of an agent which stimulates the formation of normal red blood cells. If the p-aminobenzoic acid-glutamic acid portion of PGA is represented by R, the formula for the citrovorum factor, folinic acid, is:



Pteroylglutamic acid is a yellow substance, only slightly soluble in water; its sodium salt is quite soluble in water, but both are insoluble in lipid solvents.

It is stable to heat in neutral or alkaline solution but is not stable if heated in acid media. It is inactivated by sunlight. A considerable loss occurs in foods stored at room temperature.

**Occurrence.**—It is widely distributed in nature, particularly in the foliage of plants; hence the name “folie” acid. Other good sources are yeast, cauliflower, liver, and kidney. Fair sources are beef, veal, and wheat, while root vegetables, tomatoes, bananas, rice, corn, sweet potatoes, pork, ham, and lamb contain little.

**Effects of Deficiency.**—In chicks, a lack of this factor causes anemia as well as decreased resistance to malarial infection and impairment of the response to estrogens. (Hogan and Parrott.) Rats show achromotrichia (graying of the hair) and staining of the fur and whiskers with porphyrin, while monkeys respond with a macrocytic anemia, leucopenia, diarrhea, edema, and lesions of the mouth. Curative effects of pteroylglutamic acid have been shown in anemia, leucopenia, and granulocytopenia, the two last-named conditions being characterized by a diminution in the number of leucocytes and granulocytes, respectively. These effects upon the blood led to the study of the effect of this vitamin upon various types of anemias in man.

**Clinical Uses.**—Pteroylglutamic acid seems to be quite useful in certain macrocytic anemias; that is, anemias which are characterized by the presence of giant red corpuscles in the blood. Among these are sprue, the macrocytic anemias of pregnancy, infancy, and pellagra, and those following gastric resection and other intestinal dysfunctions. In these it has been found to be an effective hematopoietic factor. (Spies.) In sprue not only does it produce satisfactory effects upon the blood picture but also relieves the gastrointestinal symptoms. It has been suggested that this group of vitamins is effective in maintaining normal gastrointestinal absorption. (Darby.)

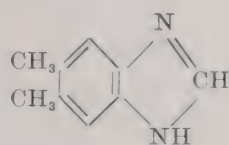
Pteroylglutamic acid also has a favorable effect upon hematopoiesis in pernicious anemia. In fact, it has qualitatively the same effect on blood formation as vitamin B<sub>12</sub>. However, far less vitamin B<sub>12</sub> is needed than PGA, and the latter is unable to check the degenerative changes in the nervous system which take place in pernicious anemia. Folie acid probably has some relation to tyrosine metabolism, possibly in conjunction with ascorbic acid.

**Requirements.**—The requirements of the human being for pteroylglutamic acid are not known at present. The dosages used in macrocytic anemias range from 10-30 mg. intravenously to 200 mg. by mouth, per day. The lethal dose for animals ranges from 125 to 600 mg. per kilogram. This is many times the therapeutic dose, and indicates the relatively low toxicity of this vitamin.

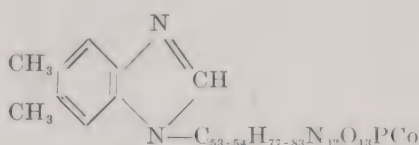
### Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub>, called the antipernicious anemia vitamin, was isolated, purified, and extensively studied in 1949. It is the factor of liver extracts responsible for the curative effects of these extracts upon pernicious anemia and it is now believed to be identical with, or very similar to, the “extrinsic

factor" of Castle. It is thought by some to be the "animal protein factor," which is also present in liver, muscle, milk products, cow manure, and other animal products. Known by several names, Factor X, Zoopherin, etc., this substance is needed to supplement yeast as a source of B vitamins for the growth of rats and is essential for many biological activities, including growth and lactation. Folkers and associates suggest it is a polyacidic base, in which the cobalt atoms are associated with cyanide groups in a coordination complex. They give it an empiric formula something like  $C_{61-64}H_{86-92}N_{14}O_{13}PCo$ . Although it contains nitrogen, it does not yield amino acids on hydrolysis. There also appears to be no pterin-type structure present. Hence it is not a folic acid derivative. One of the interesting degradation products is 5,6-dimethylbenzimidazole, the formula of which is given below, as well as the partial provisional formula for the vitamin.



5,6-Dimethylbenzimidazole

Vitamin  $B_{12}$ 

A comparison of the former with the structural formula for riboflavin (page 290) will reveal a marked resemblance. There are several vitamins  $B_{12}$  ( $B_{12a}$ ,  $B_{12b}$ ,  $B_{12c}$ , and  $B_{12d}$ ). Vitamin  $B_{12a}$  is identical with  $B_{12b}$ . Vitamin  $B_{12}$  contains a CN group and is called "cyanocobalamin." This group is not present in  $B_{12a}$ , which is known as "hydroxocobalamin." Both are therapeutically active when introduced parenterally into persons who have pernicious anemia, nutritional macrocytic anemia, and tropical sprue.

**Occurrence.**—The chief source of vitamin  $B_{12}$  is liver, although it is also present in milk, meat, eggs, fish, oysters, and clams. It has been found in the liquor resulting from the production of streptomycin, and there is some evidence that under certain dietary conditions this vitamin may be synthesized by intestinal organisms. In general, it is not present in vegetable foods.

**Properties.**—Both British and American investigators have been active in the isolation and characterization of vitamin  $B_{12}$ . It is a red crystalline compound containing nitrogen, phosphorus, and cobalt but no sulfur. Calculated on the basis of one atom of cobalt per molecule, it has a molecular weight of approximately 1,300. It is soluble in water, alcohol, and acetone but not in chloroform. It is levorotatory and is inactivated by both acids and alkalis. It has been crystallized (see Fig. 34, L).

**Clinical Uses.**—Vitamin  $B_{12}$  is perhaps the most potent therapeutic agent known. Only 1  $\mu g$  per day is required for satisfactory hematopoiesis in pernicious anemia (Jones). It is a powerful medicament in the parenteral treatment of megaloblastic anemias associated with a deficiency of the intrinsic factor. It now appears that the intrinsic factor, present in normal gastric juice, is necessary for the absorption of  $B_{12}$ , the extrinsic factor, from the gastrointestinal tract. (Berk.) Consequently, when administered by mouth,  $B_{12}$  is of little or no value unless normal gastric juice is given at the same time.



Vitamin B<sub>12</sub> not only has a curative effect upon the anemia of pernicious anemia but also on the nervous manifestations. It may also favorably influence the course of the anemias of sprue, pellagra, and infancy, but in these conditions pteroylglutamic acid may also be needed.

**Effect Upon Growth.**—If crude vitamin B<sub>12</sub> (animal protein factor) is added as a supplement to the food of young animals, a pronounced improvement in the rate of growth is observed. Since the two main constituents of the supplement are vitamin B<sub>12</sub> and streptomycin (see page 672), there has been some controversy over which one was the causative agent. Other antibiotics have also been used, often with striking results. (They have stimulating effects even upon plants.) Consequently the effects upon animals have been ascribed to (1) an improvement of appetite by the vitamin, (2) diminution of the multiplication of intestinal microorganisms by the antibiotics, and (3) specific inhibitory effects of the antibiotics upon oxidative phosphorylation. (Jukes; Black and Bratzler; Nickell; van Meter.) Whatever the mechanism, the influence of such supplements upon the growth of fowl and domestic animals may be quite important from an economic standpoint.

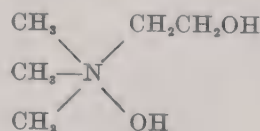
**Mechanism of Action of Folic Acid and Vitamin B<sub>12</sub>.**—The mechanism of the action of vitamin B<sub>12</sub>, folic acid, and their related substances in all of these complex relationships is not very clear. Apparently B<sub>12</sub> is necessary for the conversion of folic acid to folinic acid, a much more powerful substance. The various members of the group are concerned with the production of methyl groups, with transmethylation, or with the transfer of one-carbon groups as in the formation of serine from glycine. (Stekol; Oginsky.)

## OTHER ESSENTIAL NUTRITIONAL FACTORS

Choline and inositol do not conform to the criteria demanded by Ansbacher's definition (see page 285). They are, however, nutritionally important substances. These and one other factor,  $\alpha$ -lipoic acid, will be considered here.

### Choline

Choline is not a true vitamin because other naturally occurring substances can substitute for it and because it has not been shown to act catalytically. However, its importance in nutrition is unquestioned. It will be remembered that choline is a constituent of the lecithins and has the formula trimethylhydroxyethyl-ammonium hydroxide:



Choline

**Effect of Deficiency.**—Choline deficiency was discovered in the course of work on diabetic animals. In depancreatized dogs not only does diabetes occur, but also a fatty infiltration of the liver. The former condition may be

controlled by insulin but not the latter. Feeding raw pancreas, however, does cure the fatty liver, and the effective agent, or at least one of them, was found to be the choline part of the lecithin present in the pancreatic tissue. This so-called *lipotropic* action of choline has been demonstrated in a number of other experimentally produced fatty livers as well as in atherosclerosis. The latter is a type of arteriosclerosis in which lipid deposits occur in the arterial wall. It may be induced in rabbits by high cholesterol feeding. Administration of choline to such rabbits had a favorable effect upon the arterial condition. Application of this type of therapy to human beings was attempted with encouraging results. There was a significant reduction of mortality in coronary atherosclerosis when patients were fed large amounts of choline daily for a year or more. (Morrison and Gonzales.) Choline has been demonstrated to have a number of other physiological functions. On a low choline diet, puppies develop a severe anorexia (lack of appetite) and fail to grow; hens do not lay eggs; and rats do not have normal lactation. Together with manganese and folic acid, choline prevents perosis or "slipped tendon disease" in chicks and young turkeys, and it prevents the occurrence of cirrhosis of the liver in rats. A low choline diet also produces hemorrhages of the kidneys and eyes in addition to fatty livers in young rats, and if the diet is low in methionine as well, the hemorrhagic condition appears in other organs also.

This relationship of methionine to choline should be noted carefully. Both contain methyl groups and, with betaine, they are sources of these groups in metabolism. If methionine is added to a low choline diet, it decreases liver fat, probably because it has a methyl group to offer to the system which is lacking such groups. A shifting of the methyl groups is in some way required in fat metabolism and in other types of metabolism. This is called "transmethylation" and methyl groups are shifted, depending on the need for them and the dietary supply of them. Therefore methionine or betaine may replace choline. Some of these reactions will be considered further in later chapters.

Best and Hartroft have reported a curious effect of choline deficiency. If very young rats are kept on a choline-deficient diet for six days and are then given a normal diet, they eventually develop high blood pressure. This may indicate that some of the systemic diseases of adult life are due to unbalanced diets for relatively short periods during childhood.

As yet, no applications of choline to human nutrition have been definitely accepted. The compound is widely distributed and no deficiency need ordinarily be expected. The most important sources in our diet are cereals, bread, meat, and egg yolk.

### Inositol

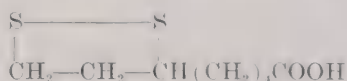
Inositol is hexahydroxy cyclohexane. The biologically active inositol—there are nine stereoisomers—is the optically inactive mesoinositol (see page 145 for formula). It is one of the muscle extractives and is also found in brain, red blood cells, and the tissues of the eye. It occurs widely in the plant kingdom in fruits, vegetables, whole grains, and nuts. Milk and yeast contain considerable amounts.

Inositol was included in the discussion of muscle extractives (page 138). It was stated that it is possibly an intermediate between aromatic substances and carbohydrates. It is found in nature in at least four forms; namely, free inositol, phytin, phosphatidyl inositol, and a water-soluble, nondialyzable complex. Phytin is the calcium and magnesium salt of inositol hexaphosphate and was formerly thought to be exclusively of vegetable origin. Now it is known to be a constituent of the nucleated erythrocytes of several species of animals. (Rapoport and Guest.) The inositol-containing phosphatide has been called "lipositol." It has been isolated in pure form from soybeans and is known to be present in brain and spinal cord. (Williams.)

There have been a number of different types of effects produced upon animals when fed a diet deficient in this factor. Among them is the observation of Woolley that inositol deficiency results in a peculiar hairlessness in mice. Gavin and associates have shown that inositol has a curative action upon the fatty livers produced in rats by the administration of biotin. Its significance in human nutrition is still unsettled, although it has been shown to be "lipotropic" for man as well as for other animals, and as a result inositol is another vitamin which is being tested therapeutically in atherosclerosis.

### $\alpha$ -Lipoic Acid

$\alpha$ -Lipoic acid is a relatively new factor (Reed). Its formula is:



$\alpha$ -Lipoic Acid  
(6,8-Dithio-n-octanoic Acid)

It appears to be necessary for oxidative decarboxylation of pyruvic acid and  $\alpha$ -ketoglutaric acid by certain microorganisms and is probably a coenzyme or part of a coenzyme for this reaction. It is found in many biological materials, including yeast and liver, and is also called the pyruvate oxidation factor (POF).  $\alpha$ -Lipoic acid has not been shown to be a dietary requirement for mammals and is not yet classed as a vitamin. (See also page 428.)

## BIOSYNTHESIS OF VITAMINS

The vitamins are synthesized to a greater or less extent in the body. This may occur as a result of bacterial growth in the intestinal canal or by metabolic transformations within the body's cells. For example, bacteria produce large amounts of vitamin K, while an instance of metabolic synthesis is seen in the tryptophan-niacin transformation (see page 291). The continued use of antibiotics per os has been found to result in vitamin deficiency signs in many cases. This was due to the destruction of vitamin-elaborating organisms in the gastrointestinal tract.

## SUBACUTE AND MULTIPLE AVITAMINOSES

The deficiency diseases due to a marked lack of a single vitamin may be termed "severe acute" conditions. Such are beriberi, scurvy, and xerophthal-



mia. Kruse clinically characterizes the avitaminoses as follows: mild acute, severe acute, mild chronic, or severe chronic. That is to say, the degree and type of the avitaminosis will depend upon the amount of vitamin lacking in the diet and the length of time it has been lacking. Fluctuations in the amount ingested (or absorbed) may account for a mild or severe acute type being superimposed on a mild or severe chronic type. These different types may respond differently to treatment. As a rule the acute types respond more quickly than do the chronic. In chronic conditions the pathological changes have been occurring for a long time and the lesions have been more permanently established.

The chronic avitaminoses are, of course, a result of the unsatisfactory nutritive condition continuing over a long period of time. Therefore, although they may appear in young people, they are more likely to occur in the aged. The longer people live on a deficient diet, the greater the chance of having these chronic symptoms develop. It is well known that the aged are more likely to have many of the ill-defined (as well as the well-defined) symptoms of malnutrition.

Since there is scarcely any food which contains only one vitamin, it is probable that most cases of avitaminosis have a lack of more than one vitamin. Even those that have the classical symptoms of a true deficiency disease usually also exhibit symptoms of other deficiencies. Pellagra is perhaps the best example because it has been shown that most pellagrins suffer from lack of most of the B vitamins while having a particular deficiency of niacin. The reason is that the unbalanced diet is deficient in several vitamins, and often in other nutrients as well. Excessive use of alcohol, dietary vagaries, or the presence of a severe fever may result in the limitation of either quantity or variety of food, or both. If a person has gastric achlorhydria, there appears to be an interference with the absorption of all the water-soluble vitamins. When biliary obstruction occurs, there may be inadequate absorption of all the fat-soluble vitamins. Thus we see how multiple avitaminoses may occur and are probably more common than single avitaminoses. Moreover, since the vitamins apparently all function as parts of enzyme systems and since these account for all metabolic activities, it is evident that the greater the total metabolism, the greater will be the requirement for vitamins; or, stated differently, the more one eats, the more vitamins one needs.

Besides noting the physical signs and symptoms which the patient shows, the physician may get additional information by utilizing the various methods which have been devised for appraising the nutritional status of the patient. These comprise biochemical, microbiological, biophysical, physical, and biomicroscopic methods. Some of the methods have been described under the vitamins concerned. They enable one to determine the vitamin level in the blood or tissues, or its excretion in the urine, or to ascertain the response to a test dose of the vitamin. Biochemical and microbiological tests are used for actual quantitative measurements of the vitamins. Biophysical methods include measurements of capillary resistance to pressure for vitamin C or "vitamin P" appraisal and the detection of difficulty in dark adaptation (vitamin A). The detection of rachitic

lesions and more especially the healing of such lesions by the x-ray is a physical procedure. The biomicroscope has been used with great success to detect, in the conjunctiva, the ocular limbus, the tongue and gums, the diverse stages of deficiencies of A, riboflavin, niacin, and ascorbic acid. It is claimed that in each case there is a specific tissue in which the pathologic process appears early and deviates in accordance with the progress of the avitaminosis. (Goldsmith.)

### VITAMINS IN DAILY LIFE

The high-pressure advertising of unethical manufacturers and distributors of vitamins has led to the indiscriminate use of vitamins by the lay public. Does vitamin deficiency account for lassitude, headaches, colds, and a multitude of other ills? Is there a lack of vitamins generally in our population?

Nutrition experts agree that under ordinary conditions the average normal adult can obtain his necessary quota of vitamins on the usual varied diet. This has become more certain since the advent of the enrichment of bread with vitamins (see page 324). Babies and young children should receive additional vitamins C and D, as discussed previously. It will be noted that the phrase "under ordinary conditions," is used. If under conditions of war, financial stress, or famine, or for any other reason, shortage of certain foods may occur, a vitamin deficit might easily result. Then the addition of vitamin concentrates might well be indicated. Individuals who are ill or who show some definite need for a particular vitamin of course must be considered in a separate class. Physicians should be particularly careful in prescribing special dietaries for patients having peptic ulcer, nephritis, etc., since these may very well be deficient in some nutrient factor, especially a vitamin. It is also to be remembered that the water-soluble vitamins are not stored to any great extent and are excreted by the kidney. Therefore they are likely to be washed away when large quantities of fluid are administered by any route and should be replenished.

### VITAMINS AS DRUGS

The vitamins, by their very definition, exert their action when present in the body in minute amounts. They must therefore be quite potent substances. They have been used in large amounts clinically in two ways: first, to rapidly make good a deficiency and second, to effect some therapeutic action. Many physiologically active compounds have two types of action; one, when given in small doses and another, often quite different, when given in large doses. It is possible that some of the vitamins likewise will have different effects in large doses. They have been tested in an enormous number of pathological conditions, sometimes singly and sometimes with other vitamins or other foods or drugs. Many of these experiments are bizarre and are tinged with the overenthusiasm of the experimenter, but some have been performed carefully and honestly. In time, no doubt, it will be found that some of the vitamins will have definite roles as drugs in addition to being food accessories.

**Toxicity of the Vitamins.**—There is little danger of toxic manifestation from the usual amounts of vitamins either in the diet or in the ordinary vitamin



concentrates. For example, the minimal lethal dose of niacin is about 6 Gm. per kilogram of body weight. For a person weighing 60 kilograms, this would amount to 360 Gm., that is, over 12 ounces, obviously an amount which no person would be at all likely to ingest. The ratio of the amount required daily for optimal nutrition to the toxic or lethal dose is about as follows: vitamin D, 1:2,000; niacin, 1:5,000; vitamin A, 1:7,500; thiamine, 1:25,000; pyridoxine, 1:60,000; and vitamin B<sub>12</sub>, 1:100,000. In view of the fact that enormous doses of some vitamins have been recommended and used clinically, it should be noted that there is a danger zone, even though it is far above the usual level of intake. There seems to be no information regarding the possible toxicity of riboflavin, pantothenic acid, p-aminobenzoic acid, or ascorbic acid. The feeding of 1,000 mg. of ascorbic acid per day to human beings for as long as three months without harmful effects has been reported. However, there have been some instances of toxic and even fatal manifestations following *repeated parenteral* large doses of thiamine.

### CONDITIONED VITAMIN DEFICIENCIES

The term "conditioned vitamin deficiency" is used to indicate those disorders which arise, not because of lack of vitamins in the diet, but because of interference with ingestion, absorption, or utilization. Some of these have been mentioned previously in various connections. They may be summarized under the following headings:

**Decreased Intake.**—Persistent vomiting from whatever cause may lead to a diminished intake. Any mouth deformities such as cleft palate or loss of teeth may have a similar effect. In chronic alcoholism there is also a diminished ingestion of all food, including vitamins. In cases of long-standing organic disease, either the appetite may be poor or the individuals may be unable to utilize their food properly.

**Impaired Utilization.**—Examples of impaired utilization of vitamins are sulfonamide therapy and malignancy.

**Decreased Absorption.**—Under this heading comes the loss of vitamins because they are (1) rushed through the enteric tract by diarrheas, or (2) dissolved in undigested "grease," as in celiac disease or after the administration of mineral oil. Defective absorption because of any damage to the epithelium or diminution in the area of absorptive surfaces as a result of operations also should be noted.

**Increased Elimination.**—Diuresis as a result of saline infusions or from other causes leads to an excessive and rapid loss of the water-soluble vitamins. Excessive perspiration and diarrhea have similar effects, and lactation, a physiological activity, may deplete the mother while benefiting the baby.

**Increased Requirements.**—Besides those increased requirements due to physiological needs, such as growth in children, pregnancy, and lactation, there are some other factors which may be mentioned. Greater utilization of carbohydrate demands greater amounts of thiamine and the other B vitamins. Thus, the infusion of glucose may lead to symptoms of B complex deficiency, as may



also sudden increase in carbohydrate with insulin administration in diabetic diets. When glucose infusions are given, concentrates of B complex vitamins should be administered concurrently. Fever increases the rate of metabolism and hence the vitamin requirement.

**Faulty Bacterial Synthesis.**—It is now known that vitamin K, several members of the B vitamins, and perhaps others are synthesized by intestinal microorganisms and may thus add a significant contribution to the vitamin intake. If the flora is not suitable, or if intestinal antisepsis is practiced (as is often the case in the oral administration of some antibiotics or sulfa drugs), a vitamin deficiency is likely to occur. (Elvehjem.)

**Bacterial Destruction.**—There is considerable evidence that a large number of intestinal bacteria can decompose ascorbic acid. Decomposition is rapid and complete but is inhibited by the presence of any sugar which the organism can ferment. Niacin, and possibly thiamine and folic acid, is also susceptible to destruction by intestinal flora.

**Inhibitors.**—The sulfonamides are direct inhibitors of p-aminobenzoic acid. Other "structural analogues" have been produced and are discussed in Chapter 24.

**Imbalance.**—There is some evidence that the amounts of the various vitamins in the diet should bear some relationship to each other. For instance, vitamin E has a sparing action on vitamin A, and instances of interdependence of the members of the B complex have also been observed.

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## Chapter 13

### FOODS

Food is needed to provide the fuel for our energy requirements, to build and rebuild tissues, and to furnish the vitamins and inorganic compounds which are indispensable to good health. Thus we eat to live, but the complementary "we live to eat" is not to be lightly regarded simply because it implies enjoyment of food. Such pleasure, we know, leads to better digestion. As a rule it also leads to a wider choice of foods, tending to provide a diversity of all the important food factors. The gourmet never dies of a deficiency disease.

#### THE ENERGY FACTOR

The energy or caloric value of foods is measured in large calories. A large calorie (Cal.) is equal to 1,000 small calories and is therefore the amount of heat required to raise the temperature of 1,000 grams of water from 15° to 16° C. In this discussion only the large calorie will be used. The common foodstuffs yield, when burned in the body, approximately the following caloric values:

	CAL./GM.
Proteins	4
Carbohydrates	4
Fats	9

These are round (and corrected) numbers. Individual members of each class have slightly different values, but these figures are usually accepted as averages and reasonably accurate.

In Chapter 21 the energy requirements of man will be discussed in greater detail. At this time it may be stated in a general way that normal men require from 2,500 to 3,000 Cal. per day at light to moderate activity and from 3,000 to 5,000 Cal. per day when doing hard work. Women require a somewhat smaller calorie intake than men, from 2,100 to 3,000 Cal., but during lactation the requirement is nearer 3,000 Cal. Children and adolescents need about the following amounts:

AGE (YR.)		CAL.
1-3		1,200
4-6		1,600
7-9		2,000
10-12		2,500
13-15	(Girls)	2,800
13-15	(Boys)	3,200
16-20	(Girls)	2,400
16-20	(Boys)	3,800

The energy factor plays a role in growth as well as in the more apparent energy relationships discussed in Chapter 21. Handler and co-workers have

shown that when animals are fed a diet in which the proteins, vitamins, and minerals are adequate, but the calories severely restricted, both skeletal and generalized body growth may be inhibited even to the point of complete cessation. Obviously, cell division, ossification, and other growth processes involve energy transformations.

**Calculating the Energy Value of a Food.**—Few naturally occurring foods are pure protein, carbohydrate, or fat. Most of them are mixtures, and, in addition, contain varying amounts of water. Their energy value is calculated on the basis of the actual percentages of protein, carbohydrate, and fat present multiplied by the caloric values given. For example: White bread contains 36 per cent water, 9 per cent protein, 2 per cent fat, 52 per cent total carbohydrates. A slice of this bread weighs about 25 grams and therefore contains

	25 × 9 per cent	25 × 2 per cent	25 × 52 per cent
	or	or	or
	2.3 Gm. protein	0.5 Gm. fat	13 Gm. carbohydrate
	× 4	× 9	× 4
Its caloric	<hr/>	<hr/>	<hr/>
value would be	9.2 Cal.	4.5 Cal.	52 Cal. = 65.7 Cal.

The approximate energy value of any food may be estimated by this type of calculation, if the composition of the food is known. The analyses of many foods are available (see Table LII) and can be found in various government publications as well as biochemical and nutritional textbooks. It must be noted, however, that all foods vary considerably from time to time. Meats will differ markedly in their fat content—even meats of the same grade and cut. A given vegetable—peas, for instance—will show differences in composition (both organic and inorganic), depending on variety, moisture availability, amount and type of fertilizer, and other variables in cultivation. Prepared foods will show the widest divergence, of course, because of the variety of ingredients and methods of cooking. It is evident, therefore, that such data must be used with intelligence, and calculation to even the first place of decimals is sheer nonsense. In Chatfield and Adam's table the energy values are given per "100 grams" and per "pound." In Hawley and Carden's tables, from which Table L is taken, all of the constituents, except the vitamins, are expressed as grams per 100 grams of edible material. The vitamins are indicated in various ways, depending upon the individual factors. Other tables are arranged to give the number of grams in average portions, the size of each portion being stated. Still others state the number of grams of each food in a 100 Cal. portion.

## THE PROTEIN FACTOR

The question as to what is the optimum amount of protein has been discussed for many years. Indeed at one time it was one of the most important topics in physiological chemistry. The reasons for this are very evident. Protein is the most important foodstuff. The caloric needs of the body can be supplied by protein, carbohydrate, and fat, but the protein of our tissues can



only come from the protein of food. Protein is required by every cell and is the basis of protoplasm. Moreover, it is the most expensive of the foodstuffs. It was therefore felt to be of economic, as well as of physiological, interest to determine the minimum protein requirement. In the latter part of the nineteenth century Voit studied the protein intake of many adults in Germany and found that their average consumption was 118 Gm. This was set up as the standard, even the optimum protein intake, although the only evidence for such an assumption was that it was the amount usually eaten. Chittenden in 1904 came to quite a different conclusion. As a result of nutritional investigations upon students, instructors, soldiers, and others, he found that the protein requirement was much less than Voit's standard. The general method used in these studies was to determine the nitrogen balance; that is, a comparison between the nitrogen intake as protein and the nitrogen output in the urine and feces. If these are equal the subject is in nitrogen equilibrium. If the output is greater than the intake, there is a negative balance, which means that the individual is not getting enough protein in his food to take care of his physiological requirements. Chittenden's subjects could be kept in nitrogen equilibrium on from 45 to 53 Gm. of protein daily. The subjects on low protein diets were able to do just as much physical and mental work as before. In fact, it was reported that their general health and athletic prowess were usually improved. Chittenden came to the conclusion that Voit's standard of 118 Gm. per day was far above the actual needs of men and that the excess intake might actually lead to various pathological states; e.g., indigestion, intestinal toxemia, liver trouble, gout, and rheumatism. Many investigations have shown that the minimum protein requirement compatible with nitrogen equilibrium is much lower even than the figures set by Chittenden. In order to attain nitrogen equilibrium with a low protein intake, large quantities of carbohydrate and fat must be eaten to provide enough energy for the individual's needs. If this is not done, body protein will be consumed for this energy requirement, and more nitrogen will be eliminated than is taken in. Consequently, carbohydrates and fats are known as "protein spacers." The question has been raised as to whether a person could continue on a very low protein diet for any considerable length of time, even if he was in nitrogen equilibrium. All experiments have necessarily been restricted to a few weeks or, at best, a few months. But could a person live for years, and live comfortably, on a minimum or near-minimum protein ration? Nutritionists today doubt if that could be accomplished. With the modern conception of "essential amino acids," it becomes apparent that a person on a highly restricted diet might easily experience a lack of one or more of these vital building stones. If the protein or amino acid content of the diet could be so planned as to have *all* the essential amino acids in *requisite amounts*, at about the same time, such a lack would not occur, and a minimal protein diet might be the ideal one. However, that is not possible at present, even if it were demonstrated to be entirely desirable. It would be practically impossible to select foods having exactly the same amino acid composition as human proteins so that food protein could be converted into body protein weight for weight.

Consequently an amount of protein greater than the Chittenden figure, and yet lower than the Voit figure, is today considered the optimum one for good nutritive condition. Sherman and co-workers point out that the protein *requirement* of man is quite different from the protein *allowance* or *standard*. Total calories need must not exceed total calories required by very much or for very long. Such excess is usually converted into fat. However, excess protein over the required minimum is not stored to any great extent, and is not converted into fat, unless the total caloric value of the food is excessive. Therefore, a moderate excess of protein over and above the required amount is not only harmless but is a common dietary habit which constitutes a desirable "factor of safety." It provides an excess of indispensable and semindispensable amino acids (page 406) which circulate in the blood or are temporarily stored in the tissues, ready and available for any cell in need of them.

The studies of various investigators have shown that an intake of about 1 Gm. of protein per kilogram of body weight is adequate for all ordinary needs in the normal adult. In childhood this allowance must be increased considerably to permit of growth of new tissue. The same applies in pregnancy and lactation. In elderly people a lower requirement is noted along with lower caloric need. Contrary to popular opinion, the protein requirement does not run parallel to the amount of work done. The total calories of the diet, of course, do, but the body protein is not used up appreciably faster by hard work than by little muscular activity.

The quality of the protein eaten is an important factor in nutrition. In general the proteins of eggs, milk, kidneys, and liver are the best biologically. Eggs are in first place. They contain the highest percentages of the essential amino acids. Meats, poultry, and fish, as well as shell fish, come next; in the same class are yeast and soybeans. Cereal, nut, and legume proteins are generally poor. Moreover, meat proteins have a high "coefficient of digestibility," which means that the amino acids are readily split off by the enzymes and are available for absorption. From this standpoint kidney is superior to other meats, liver next, and muscle meats third. Vegetables are less easily digested, perhaps only 80 per cent as well. As a rule, however, one does not eat one food exclusively for a long period of time. When this does occur one may expect unhappy consequences. Thus a diet consisting largely of corn is quite likely to cause pellagra, because the corn proteins are deficient in tryptophan, as well as lysine (see Table XXVI). The corn proteins plus milk, meat, eggs, or soybeans would constitute an admirable set of proteins from a biological standpoint. It is most fortunate that milk proteins are entirely adequate for growth, since milk is the sole diet of infants for a long time.

For human nutrition most of the varied dietaries of modern civilization provide for adequate mixtures and amounts of the essential amino acids. Even when the cereal grains, especially wheat and rye, furnish most of the protein intake, only one amino acid, lysine, is likely to be lacking. It has been suggested by Block that the enrichment of wheat breads with lysine would more than double their biological value, converting them from inferior protein foods to foods having almost the nutritive value of the more expensive animal protein



foods. Corn, soybean, and food yeast have been found to be a nutritionally adequate mixture, as has refined wheat flour and yeast (Odendaal; Sure). Such plant mixtures might conceivably be substituted for meat, in part at least, or entirely for short periods, in emergencies. In animal nutrition, where herds of cattle and flocks of poultry are fed the same foodstuffs for long periods of time it is easy to understand how specific amino acid deficiency might occur. Animals fed cereals alone are likely to be poorly nourished because of a deficiency in some of the essential amino acids. A supplement of skim milk will remedy this defect. In Table XXVI is shown the distribution of amino acids in some of the common food proteins, and it is quite apparent that the foods vary considerably in their amino acid composition.

TABLE XXVI  
APPROXIMATE PERCENTAGE OF AMINO ACIDS IN SOME ANIMAL AND VEGETABLE PROTEINS  
(CALCULATED TO 16.0 PER CENT NITROGEN)\*

	WHOLE MILK		CASE- IN (%)	WHOLE EGG (%)	EGG WHITE (%)	MEAT (%)	FISH (%)	GELA- TIN (%)	WHOLE GRAINS		PEA- NUT (%)	SOY- BEAN (%)
	HU- MAN (%)	COW'S (%)							CORN (%)	WHEAT (%)		
Arginine	5.0	4.3	4.1	7.0	5.8	7.1	5.6	7.6	4.0	3.0	9.9	5.8
Histidine	2.7	2.5	2.5	2.4	2.2	2.2	1.9	1.0	2.4	1.2	2.1	2.3
Lysine	7.2	7.5	6.9	6.0	6.5	8.1	6.8	4.3	2.5	2.7	3.0	5.4
Tyrosine	5.1	5.3	6.4	5.0	4.8	3.1	4.0	0.2	6.1	3.8	4.4	4.1
Tryptophan	1.9	1.6	1.5	1.6	1.6	1.2	1.3	0.0	0.6	1.0	1.0	1.5
Phenylalanine	5.9	5.7	5.2	5.6	5.5	4.5	4.5	1.8	4.5	5.7	5.4	5.3
Cystine	3.4	1.1	0.4	2.1	2.3	1.1	1.2	0.1	1.1	1.3	1.6	0.6-1.1
Methionine	2.0	2.8	3.5	4.0	4.4	3.3	3.4	0.8	4.0	3.0	0.9	1.8
Threonine	4.6	4.6	3.9	4.9		4.3	4.4	1.5	3.6	3.3	1.5	4.0
Leucine	15.0	15.0	12.0	19.0		12.0		4.0	22.0	13.0	10.0	8.0
Isoleucine	5.2	4.4	5.0	5.3		3.4		1.0	3.4	4.0	3.4	4.0
Valine	5.5	5.0	7.0	4.5		3.4		2.0	5.0	3.4	7.0	4.5
Glycine			0.5	2.7	3.3	5.0		23.6			5.6	

\*Data from Block, R. J., and Bolling, D.: J. Am. Dietet. A. 20: 69, 1944.

Heat processing alters the nutritive value of proteins. Egg white is said to be more easily digested, and phaseolin, a protein of the navy bean, has greater nutritive value if cooked. Soybeans have a higher nutritional value after cooking. This is due, in part, to the inactivation and detoxication of a toxic protein, soyin (Liener and Pallasch). As mentioned on page 217, there is also present in soybeans a trypsin inhibitor, which is destroyed by heat (Ham and Sandstedt). *Dry heat*, however, seems to have a deleterious effect, particularly upon the proteins of cereals. Apparently lysine becomes useless although it is not destroyed. It is thought that a linkage between the epsilon amino group and a free carboxyl group of a dicarboxylic amino acid results in the formation of an unnatural peptide linkage which cannot be split by the digestive enzymes. (Mitchell and Block.) Other explanations have been suggested. One is that, as a result of heating, the protein is so altered that the rate of release of the particular amino acids is slowed up. The delays result in less



efficient amino acid mixtures. (Melnick and Oser.) The importance of these observations lies in the widespread use of toasted and "puffed" cereals in the American dietary.

By several experimental methods it has been shown that carbohydrate can be formed from protein. If animals are made diabetic, either by injecting phloridzin or by removing the pancreas surgically, this transformation can easily be demonstrated. After such animals have been depleted of their glycogen and fat stores, the feeding of protein alone leads to the excretion in the urine of glucose in amounts greater than that excreted previously. Since the nitrogen of the urine originates chiefly in the protein of the diet, it would seem that one could calculate the exact amount of sugar which is derived from protein by studying the output of glucose and nitrogen in the urine of such diabetic animals. In fact, this has been done and the glucose:nitrogen ratio (formerly called D:N; i.e., dextrose:nitrogen) has long been a classic method for such calculations. In phloridzinized dogs the glucose:nitrogen ratio was said to be 3.6:1. Since the nitrogen content of protein is about 16 per cent, this is equal to 58:16; that is, every 100 Gm. of protein would lead to the excretion of 58 Gm. of glucose and 16 Gm. of nitrogen. Recently it has been pointed out that there are many sources of inaccuracy in using the glucose:nitrogen ratio and consequently it is probably not entirely correct to say that 58 per cent of protein is convertible into carbohydrate. This, however, is the figure that nutritionists and dieticians have used and will use in calculating the glucose equivalent of diets until a more accurate figure is demonstrated. For example, it is employed in planning the diet of a diabetic individual when the total amount of carbohydrate present plus that derivable from protein is wanted in order to calculate the insulin requirement.

The source of the glucose obtained from protein is of course the carbon chains. Some of the carbon is used, as will be seen, together with the nitrogen to form urea. Much of the remainder should be available for carbohydrate formation. All amino acids, however, do not form glucose; some form acetoacetic acid (see Chapter 15).

## THE CARBOHYDRATE FACTOR

Glucose, fructose, galactose, and, to a minor degree, mannose, as well as those carbohydrates which yield them on digestion, are available to the body as energy producers. The pentoses in foods seem to be of no value nutritionally except those that are in the nucleoproteins or nucleotides, and the utilization of these is not well understood at the present time. However, they form a very small fraction of the total carbohydrate intake. As stated before, polysaccharides or even disaccharides cannot be utilized until digested to the monosaccharide stage. When introduced directly into the blood stream, they act as foreign bodies and are excreted, chiefly by the kidneys.

Other nonutilizable carbohydrates are the indigestible polysaccharides—cellulose, lignin, agar-agar, gums, etc. These constitute a large part of the roughage of the food, the indigestible fraction which gives bulk to the feces. It is quite essential that food contain such substances, since a dearth of them

tends to produce constipation. The tendency in modern civilization has been toward a refinement of food, with a lessening in the amount of the indigestible parts of grains, fruits, and vegetables. On the other hand, an overabundance of roughage may lead to irritation of the intestinal mucosa. It is not entirely the physical presence of the indigestible material, or the distention which it causes, that stimulates peristalsis. Certain intestinal bacteria are capable of decomposing hemicelluloses and mixed polysaccharides, with the production of lower volatile fatty acids. These, together with the hygroscopic nature of these carbohydrates and of some of the other products resulting from the action of microorganisms, have a stimulating effect and so produce bowel movement. Lignin and cellulose, which also escape digestion, have less effect than the hemicelluloses and mixed polysaccharides in this respect. It is thus evident that the total carbohydrate of a food is not available for energy by the body. In food tables this fraction is usually termed "fiber"; at any rate, much of it comes under that heading.

The monosaccharides which are utilized are converted to glucose after absorption. The glucose then is either utilized directly, or indirectly, after it has been converted to glycogen or to fat. It is in these two forms that excess carbohydrate is stored in a number of tissues and organs. Since some part of the protein molecule is transformed into sugar, this also may become part of the glycogen and fat stores of the body.

### THE FAT FACTOR

Fat furnishes a proportionately greater amount of the body energy than do the other two foodstuffs. It is more slowly digested, and large amounts of it in a meal tend to slow down the digestion of the other foods as well. Furthermore, fats are usually not quite as completely digested as the digestible carbohydrates and proteins. This is probably not because of any inherent indigestibility of a particular fat but because of insufficient amount of lipase present, because the conditions for fat digestion are not entirely favorable, and because much of the fat is absorbed, undigested, in emulsified form.

There is no rule for the proportions of fat and carbohydrate in a diet. The normal person can get along equally well on a diet high or low in either fat or carbohydrate. Of course the "essential fatty acids" must be supplied (see page 463), but the requirement is small and these fatty acids are found in many food oils. Dietary fat has a rather high "satiety value"; that is, the ability to satisfy hunger. Furthermore, fat has a greater effect than carbohydrate or protein in determining the specific dynamic action of foods. (See page 570.)

It is common knowledge that carbohydrate can be converted to fat, and some of the protein also may be, after first passing through the sugar stage. But can fat be converted to carbohydrate? It is generally agreed that the glycerol fraction of fat can be, since it is a simple three-carbon chain. For purposes of dietetic computation this is usually taken as 10 per cent of the average fat molecule. Since fatty acids are catabolized to the two-carbon



age, and then can enter the citric acid cycle and other metabolic systems, is evident that the possible conversion of fatty acid to sugar is no longer a subject of controversy. It will be discussed further in Chapter 17.

### THE MINERAL FACTOR

The functions of the individual ions or elements will be discussed in Chapter 18. Here the chief mineral elements which are of importance in foods will be pointed out. These are calcium, magnesium, sodium, potassium, phosphorus, chlorine, sulfur, iron, and iodine.

Foods which are relatively high in calcium salts are milk and milk products, beans, leafy vegetables, and shell fish. Vegetables, in general, contain more calcium than animal foods. Some vegetables, however, have an appreciable amount of oxalic acid present, and this will form insoluble calcium oxalate in the intestinal tract and thus prevent the absorption and utilization of some of the calcium present. These foods include rhubarb, Swiss chard, spinach, beet greens, cocoa, and tea. Moreover, although calcium is widely distributed, it is not present in most foods in sufficiently high concentration, and a deficiency of this element in our diet is frequently evident. A temporary deficiency or even a long-continued deficiency of calcium in the food may occur without the appearance of any symptoms attributable to a low calcium metabolism because of the fact that the bones act as a storehouse of the element. Under the influence of parathormone, the principle elaborated by the parathyroid glands, this element is withdrawn from the bone to make good the losses from the blood and soft tissues. The minimal adult requirement has been found to be about 0.5 Gm. of calcium per day, but, since some of the calcium may be lost as calcium oxalate and other insoluble calcium compounds, a higher level is usually advised. The recommended amount for adults has been set at 1.0 Gm. and for children, at 1.0 to 1.4 Gm. In pregnancy at least 1.5 Gm. and during lactation 2.0 Gm. are required to provide the necessary calcium salts for the growing fetus and for the high calcium content of the milk, respectively. Since a quart of cow's milk contains about 1.2 Gm. of calcium, one can see that a pint of milk a day will go far toward providing the total calcium requirement of an adult and a quart of milk will do the same for a child. The importance of milk in the dietary, from this standpoint, cannot be stressed too strongly.

Phosphorus is found in those foods containing phosphoproteins, phospholipids, and glycerophosphates, as well as the inorganic phosphates, which are chiefly calcium and sodium phosphates. Since, quantitatively, the greatest proportion of the phosphorus is used to form the bone salt, which is largely calcium phosphate, it is evident that the phosphorus intake should bear an optimum relationship to the calcium intake. That is why the expression Ca:P ratio is used so often in this connection. In growing children the Ca:P ratio should be between one and two. That is, the calcium intake should be as great as the phosphorus, or greater—up to twice as great. In the adult, the need for bone formation being less, the Ca:P ratio may be lower. The particular foods which are richest in calcium are also richest in phosphorus; namely,



milk, cheese, and beans. Eggs, cereals, fish, and meats are also high in this element. If the calcium intake is satisfactory, the phosphorus will also usually be.

The common mixed dietary contains sufficient magnesium, sodium, and potassium. These elements are widely distributed in foods, and, unless too much highly refined food, such as white flour, is consumed, there is scarcely a likelihood of a deficiency. The same applies to the chlorine. Since we add sodium chloride to our foods, we ordinarily have enough of both elements. The sodium and potassium content of many articles of diet may be found in Table LI of the appendix. When there is excessive perspiration, as in summer, or in the case of men working at high temperatures, as at blast furnaces, there may be a need for additional salt intake. This is generally recognized by physicians and industrial experts. Magnesium is needed for bone salts, and the proper balance of sodium, magnesium, calcium, and potassium is of importance in regulating the irritability of many, if not all, cells. Chlorine is required to produce gastric HCl, and it also plays a role in the transport of gases in the blood.

We obtain most of our sulfur from sulfur-containing amino acids, methionine, cysteine, and cystine. There is a small amount of organic sulfide in foods but very little sulfate. The organic sulfur is oxidized in large part to sulfate and as such plays a significant role in acid-base balance. One of the sulfur-containing amino acids, methionine, is considered essential. Other sulfur-containing compounds of physiological importance are glutathione, insulin, biotin, and thiamine. The supply of sulfur in our food is chiefly dependent upon the amount and quality of the protein. Therefore an optimum protein diet will lead inevitably to an adequate sulfur intake.

Iron is, of course, bound up with hemoglobin formation. It is therefore necessary to have a sufficient quantity of iron available whenever new hemoglobin must be produced. Children, invalids, or patients, who have lost blood, naturally are in greater need of iron than are normal adults. Many foods are at hand that have considerable quantities, but there is some doubt as to what type of iron can be utilized by the body. Some authorities maintain that iron in the heme form is of no value, that it cannot be absorbed and utilized, and that only iron in the ionizable form is of physiological value. If this is true, the total iron content of any food does not necessarily represent the amount available. All nutrition authorities are not in agreement on this point. It has been shown, for example, that the iron in beef is made available by merely heating it and it is quite possible that further investigation will show that much of the iron of the heme and related forms can be rendered physiologically available in this way.

Iodine is needed in very small amounts by man, but there is no general agreement as to the exact requirement. The estimates vary from 14 to 30 gamma per day, but even the highest figure is still a very minute amount. Nevertheless these minute amounts may be lacking in diets, particularly in regions remote from the ocean. Sea foods in general have a satisfactory content of iodine, and vegetables and fruits grown on the seaboard, or wherever

he soil contains iodine, likewise are sufficiently rich in this element. In sections where a low iodine content of the diet occurs, sodium or potassium iodide is generally added to the public drinking water or to the table salt (1:5,000 to 1:200,000).

Other elements which are also present in minute amounts in foods are fluorine, bromine, copper, zinc, manganese, silicon, aluminum, cobalt, and nickel. Copper, cobalt, zinc, manganese, and, perhaps, fluorine in traces are essential to man, but the others are accidental constituents and are of no particular benefit and of no harm.

### Acid- and Base-Forming Properties of Foods

When a food is incinerated in a crucible, the ash remaining will have an acid, alkaline, or neutral reaction, depending upon the proportion and type of anions and cations present and the effect of heat upon them. When the same food is consumed by a person, its final products will sometimes have the same reaction as the ash, but there are other factors which modify the "ash" left by vital processes. Proteins and nucleoproteins yield sulfuric, phosphoric, and uric acids. These acids are neutralized by basic elements before excretion and thus tend to diminish the alkaline factors of blood and urine. Fruits and vegetables usually have enough positive radicals, such as calcium, magnesium, sodium, and potassium, to combine with the acid produced by proteins or with other acids. Organic acids such as citric, malic, tartaric, and lactic, present in fruits and vegetables, are oxidized to carbon dioxide. Most of this is lost by way of the lungs, while the potassium salts of the above acids, also occurring in fruits, are oxidized to  $\text{KHCO}_3$ , which, if present in excess, is excreted in the urine. Thus vegetables, even acid fruits, usually have an alkaline effect. There are some exceptions. For example, benzoic acid pres-

TABLE XXVII  
POTENTIAL ACIDITY OR ALKALINITY OF FOODS

FOODS HAVING A PREDOMINANTLY ACIDIC EFFECT		FOODS HAVING A NEUTRAL OR NEARLY NEUTRAL EFFECT	FOODS HAVING A PREDOMINANTLY BASIC EFFECT	
Almond	Ham	Butter	Apples	Grapes
Baking powder	Lamb	Cottonseed oil	Apricots	Lemons
Biscuit	Liver	Cream	Asparagus	Lettuce
Barley	Lobster	Custard	Bananas	Oranges
Beef	Macaroni or spaghetti	Fudge, chocolate	Beans, lima	Peaches
Bread, rye	Oysters	Honey	Beans, string	Pears
Bread, white	Pastry	Ice cream	Broccoli	Pineapple
Cake, plain	Plums*	Milk, whole	Brussels sprouts	Potatoes, white
Cake, cheese	Pork	Milk, butter	Cabbage	Potatoes, sweet
Cheese	Prunes*	Olive oil	Cantaloupe	Raisins
Corn	Sausage	Onions	Carrots	Raspberries
Cub	Scallops	Pie, apple	Cauliflower	Tomatoes
Cucumbers	Shredded wheat	Sugar	Celery	Walnuts
Raspberries*	Shrimp	Syrups	Dates	
Tea	Turkey	Tapioca	Eggplant	
Veal			Figs	
			Grapefruit	

\*The ash of these foods is alkaline but, because of the presence of benzoates, which form benzoic acid, they increase the acidity of the urine.

ent in cranberries is not oxidized by the body and is excreted as hippuric acid (after combining with glycine) and thus has an acidie effect upon the urine. Oxalic acid found in rhubarb, beet leaves, cocoa, and tea is also an exception. This is oxidized very poorly and is neutralized and excreted as oxalate. (See Table XXVII.)

### Vitamins in Foods

Although vitamins have been discussed in some detail in Chapter 12, a few points may be added or repeated here from the standpoint of foods. It is true that under normal conditions the average varied diet contains sufficient vitamins of all kinds for normal, growing children and adults. (Babies, however, require additional vitamins C and D to supplement their milk diet.) It would seem, therefore, that there should be little need for vitamin concentrates in good health. The "normal conditions" mentioned, however, mean access to a sufficient amount of fresh foods, properly cooked, and in a suitable variety. Absolutely fresh vegetables are difficult to obtain in the cities, and storage causes a progressive loss of vitamin C. Very often in the preparation of vegetables in restaurants, hotels, and hospitals, they are kept on the steamtable for hours. Under these conditions some of vitamins A, B, and C may be oxidized and lost. If the vegetables are drained of their cook water, all of the water-soluble vitamins suffer some loss by being thus discarded. Even the shredding or cutting of raw vegetables served as salad results in the liberation of ascorbic acid oxidase and the oxidation of a considerable amount of ascorbic acid. Meals, therefore, have a higher content of vitamins if they are carefully prepared and served promptly. In general, however, almost the only vitamin that we are likely to lack under conditions of a varied diet is thiamine, vitamin B<sub>1</sub>. It is not as widely distributed as the other water-soluble vitamins and, being water soluble, is not stored to an appreciable extent.

The milling of wheat results in the production of a refined white flour, which has greater keeping qualities than whole wheat flour. The loss of the germ and bran, however, reduces its thiamine, niacin, riboflavin, and iron content tremendously, as well as its calcium, phosphorus, fat, and protein to some extent. This is now partly remedied by the "enrichment" of white flour, which was accomplished during World War II by Federal order and is now carried out by millers under state laws and also voluntarily. Table XXVIII shows that enriched bread compares favorably with whole wheat bread in vitamin and mineral constituents but is still slightly deficient in protein.

TABLE XXVIII  
COMPARISON OF ENRICHED, PLAIN WHITE, AND WHOLE WHEAT BREADS\*

	PLAIN WHITE BREAD	ENRICHED WHITE BREAD	WHOLE WHEAT BREAD
Thiamine, mg. per lb.	0.3	1.1-1.8	1.3
Riboflavin, mg. per lb.	0.5	0.7-1.6	0.7
Niacin, mg. per lb.	3.0	10-15	16.0
Iron, mg. per lb.	3.9	8-12.5	11.8
Calcium, mg. per lb.	254	254†	272
Protein, gm. per lb.	39	39	43

\*From United States Department of Agriculture, Bull. AIS-39.

†Enriched bread may contain 300-800 mg. of calcium per pound as well as 150-750 U. P. units of vitamin D.



Following the introduction of enriched flour many other foods have had vitamins or minerals, or both, added to them. Among these are corn meal and grits, macaroni and spaghetti, rice, and processed cereals. Hydrogenated fats are devoid of vitamin A because hydrogenation inactivates the vitamin. For this reason oleomargarine is commonly fortified with at least 9,000 U. S. P. units of vitamin A per pound, and much of the milk on the market has additional vitamin D added to it.

Under extraordinary circumstances, such as food rationing or other conditions of food shortages, vitamins may be lacking to a greater degree. "One-sided" diets, because of allergies, or because of some other pathological condition, may also lead to vitamin deficiencies. This sometimes occurs when an individual, because of extreme fondness or violent dislike for particular foods, constantly restricts himself to a very few items of diet. If these conditions cannot easily be remedied by vitamin-rich foods, the vitamin concentrate indicated should be resorted to. Some physicians believe that patients with low resistance are benefited by a "shotgun" concentrate of four or five of the most important vitamins. At present we know of no contraindications.

### Recommended Dietary Allowances

The Food and Nutrition Board of the National Research Council has collected the data of recommended allowances of the calories, protein, calcium, iron, and vitamins. They are presented in Table XXIX. These figures represent not the minimum requirements but the amounts which, according to present information, should be ample for good nutritive conditions. No absolute requirements can be set for any factor. All are relative and usually refer to the average person on an average diet. In general they furnish a considerable margin of safety; that is, they are somewhat higher than nutritionists generally feel are absolutely necessary. The Canadian Council on Nutrition suggests lower values for several vitamins; that is, 3,000 instead of 5,000 I.U. of vitamin A, 0.9 mg. instead of 1.5 mg. of thiamine, and 1.5 mg. instead of 1.8 mg. of riboflavin, for a man weighing 70 kilograms.

**"The Basic Seven."**—For convenience in planning the dietary, especially for the housewife with little knowledge of scientific nutrition, the classification of foods into "the basic seven" has been devised. This is a broader grouping than might be desired, but it is easy to remember and should accomplish its object. "For health," we are told, "eat some food from each of the basic seven food groups every day." These seven groups are as follows:

1. Milk—two or more glasses daily for adults; three or four or more glasses daily for children
2. Vegetables—two or more servings daily besides potato; one raw vegetable; green and yellow, often
3. Fruits—two or more servings daily; one citrus fruit or tomato
4. Eggs—three to five a week; one daily preferred
5. Meat, cheese, fish, or legumes—one or more servings daily
6. Cereal or bread—most of it whole grain or "enriched"
7. Butter—two or more tablespoons daily

**Condiments.**—Among condiments we may class table salt, vinegar, spices, ketchup and other sauces, and flavoring extracts. These are the substances

TABLE XXIX

## RECOMMENDED DAILY DIETARY ALLOWANCES, REVISED 1954\*

(Allowances Are Considered to Apply to Persons Normally Vigorous and Living in Temperate Climate)

	AGE (YR.)	WEIGHT KG. (LB.)	HEIGHT CM. (IN.)	CALORIES	PROTEIN (GM.)	CALCIUM (GM.)	IRON (MG.)	VITAMIN A (I.U.)	THIAMINE (MG.)	RIBO- FLAVIN (MG.)	NIACIN (MG.)	ASCORBIC ACID (MG.)	VITAMIN D (I.U.)
Men	25	65 (143)	170 (67)	3200 <sup>1</sup>	65	0.8	12	5000	1.5	1.6	15	75	
	45	65 (143)	170 (67)	2900	65	0.8	12	5000	1.3	1.6	13	75	
	65	65 (143)	170 (67)	2600	65	0.8	12	5000	1.1	1.6	11	75	
Women	25	55 (121)	157 (62)	2300 <sup>1</sup>	55	0.8	12	5000	1.2	1.4	12	70	
	45	55 (121)	157 (62)	2100	55	0.8	12	5000	1.0	1.4	10	70	
	65	55 (121)	157 (62)	1800	55	0.8	12	5000	1.0	1.4	10	70	
	Pregnant (3rd trimester)			Add 400	80	1.5	15	6000	1.5	2.0	15	100	400
	Lactating (850 ml. daily)			Add 1000	100	2.0	15	8000	1.5	2.5	15	150	400
Infants <sup>2,3</sup>	1/12-3/12	5 (11)	58 (23)	kg. × 120	kg. × 3.5	0.6	6	1500	0.3	0.4	3	30	400
	4/12-9/12	8 (18)	67 (26)	kg. × 110	kg. × 3.5	0.8	6	1500	0.5	0.7	5	30	400
	10/12-1	10 (22)	75 (30)	kg. × 100	kg. × 3.5	1.0	6	1500	0.5	0.9	5	30	400
Children	1-3	12 (27)	87 (34)	1200	40	1.0	7	2000	0.6	1.0	6	35	400
	4-6	18 (40)	109 (43)	1600	50	1.0	8	2500	0.8	1.2	8	50	400
	7-9	27 (59)	129 (51)	2000	60	1.0	10	3500	1.0	1.5	10	60	400
Boys	10-12	35 (78)	144 (57)	2500	70	1.2	12	4500	1.3	1.8	13	75	400
	13-15	49 (108)	163 (64)	3200	85	1.4	15	5000	1.6	2.1	16	90	400
	16-20	63 (139)	175 (69)	3800	100	1.4	15	5000	1.9	2.5	19	100	400
Girls	10-12	36 (79)	144 (57)	2300	70	1.2	12	4500	1.2	1.8	12	75	400
	13-15	49 (108)	160 (63)	2500	80	1.3	15	5000	1.3	2.0	13	80	400
	16-20	54 (120)	162 (64)	2400	75	1.3	15	5000	1.2	1.9	12	80	400

\*National Research Council, Food and Nutrition Board, *Recommended Daily Dietary Allowances*, Revised 1954, Washington, D. C.<sup>1</sup>These calorie recommendations are probably excessive for the urban "white-collar" worker. In any case, the calorie allowance must be adjusted to the actual needs of the individual as required to achieve and maintain his desirable weight.<sup>2</sup>The recommendations for infants pertain to nutrients derived primarily from cow's milk. If the milk from which the protein is derived is human milk or has been treated to render it more digestible, the allowance may be in the range of 2-3 grams per kilogram. There should be no question that human milk is a desirable source of nutrients for infants, even though it may not provide the recommended levels for certain nutrients.<sup>3</sup>During the first month of life, desirable allowances for many nutrients are dependent upon maturation of excretory and endocrine functions. Therefore no specific recommendations are given.

which add savor and zest to the meal. Their judicious use by cooks, or by diners, may transform insipid, unappetizing meals into delicious repasts. In that way they are valuable both in enabling one to make good use of foods which otherwise would be less acceptable and in stimulating the flow of digestive fluids.

Some of them have other qualities. Sodium chloride is an absolute necessity but is present in the average diet in sufficient quantities for most individuals under ordinary circumstances. Ketchup and similar sauces often contain appreciable amounts of vitamins, minerals, and small amounts of protein, carbohydrate, and fat. Vinegar is dilute acetic acid with minute amounts of flavoring substances. The spices contain volatile oils which give them their specific aroma and flavor. There are the stimulating spices, such as the peppers, mustard, and horse-radish; the aromatic spices, of which allspice, anise, cinnamon, clove, ginger, and nutmeg are examples; and the sweet herbs, which include dill, marjoram, sage, and thyme. An excess of any of the spices may cause indigestion because of irritation of the gastrointestinal mucosa. Flavoring extracts are usually alcoholic solutions of the flavor-containing parts of plants and here, too, volatile oils are, for the most part, the active ingredients. Several synthetic products have been prepared which imitate the natural ones very closely, but usually the natural flavor has impurities which give it a fuller flavor, or "bouquet." Vanilla is the most commonly used extract, with lemon, almond, wintergreen, and others also frequently used. Although some of the special constituents have some pharmacological properties, the amounts involved in cooking, baking, etc., are usually so small as to render any effects inconsequential.

**Beverages.**—Coffee and tea have little food value except for the sugar, milk, or cream added. Both contain the purine alkaloid, caffeine, which is responsible for their stimulating properties. Both coffee beans and tea leaves contain tannins and the amount present in the beverage will depend largely upon the method of preparation. The distinctive flavors arise from aromatic volatile derivatives which also have slight pharmacological action. Cocoa and chocolate have higher food value, even without the usual additions, than do coffee and tea. Cocoa has had some of the fat removed. Both contain theobromine, a purine alkaloid similar to caffeine but with less effect upon the nervous system. The cola beverages also contain caffeine. Of all of these beverages probably cocoa and chocolate are the most nutritious and least harmful. The question of whether alcohol is a food is difficult to answer. It is almost completely utilized by the body when ingested in moderate amounts and yields about 7 Calories per gram. This energy is available for heat and work and for sparing other foodstuffs. It has been thought that it cannot be converted into glycogen, but since  $\text{CO}_2$  can be utilized in the formation of glycogen (see page 417) there is no reason to assume that the  $\text{CO}_2$  resulting from the breakdown of alcohol cannot be. On the other side of the question is the fact that excessive amounts of alcohol have toxic properties not found in ordinary foods. Furthermore, it is habit forming and may cause serious pathological conditions.

Extensive damage to teeth may result from the frequent use of lemon juice (Stafne and Lovestedt) or beverages containing free acid. Certain cola beverages



ages have as much as 10 per cent phosphoric acid. These acids have been found to cause a marked deleterious effect upon tooth structure, particularly if the drink is ingested daily and at times other than with meals. However, the buffering action of saliva, combined with the fact that such acids stimulate the flow of saliva, would seem to minimize this danger, particularly if the amounts taken are not excessive.

### Preservation of Foods

That the preservation of foods is of tremendous importance needs no explanation. This has been true for centuries. The ancients stored grain in seasons of plenty to provide for days of want. Savages have preserved game and fish by drying, salting, and smoking, and some of their primitive methods are still successfully used. To these were later added pickling and the making of jellies and preserves. Within the last century refrigeration has progressed to such an extent that, with the increased cold-storage facilities for eggs, meats, and other perishable foods, it has become one of the major methods of food preservation. Canning also has become an industry of vast dimensions, and more recently the freezing and the dehydration of foods, particularly vegetables, have added new conveniences and economies to food preservation and transportation.

When meat or fish is preserved by pickling, from 18 to 25 per cent of salt is added to the fluid in which the food is soaked. The salt precipitates some of the protein and therefore hardens the fibers. A small amount of the protein is lost in the brine, but most of it is left intact. Spices are added both for flavor and to aid in preservation. Sometimes vinegar is also used, particularly when fish is pickled. The pickled or canned meats are always cooked before being eaten, and there is little danger of food poisoning or parasite infestation. Pickled fish, however, are usually eaten uncooked. The food value of such products is high, although the digestibility varies with the method of preservation employed, as well as with the individual. The same criticisms apply to smoked meats and fish. They are usually salted first and then smoked. The antiseptic effect of the smoke is due to formaldehyde, creosote, acetic acid, etc., in the smoke. Smoked meats and sausages are sometimes eaten raw, and there is danger from the ova of parasites but not much danger from bacteria. No studies on the preservation of vitamins in pickled, salted, and smoked products have been made. Many individuals have difficulty in digesting pickled, salted, or smoked foods, and they should seldom be included in a patient's diet.

Jellies and preserves are excellent sources of calories but are not recommended for children as highly as they formerly were. Fruits are cooked with sugar and spices, and the chief antiseptic action comes from the high concentration of sugar. However, the jellymaker always seals the hot fluid with paraffin to exclude organisms so far as possible. The gel formation is brought about by the interaction of pectin, fruit acids, and sugar. Preserves are not in gel form but consist of the whole or portions of the fruit cooked with sugar and spices to a thick consistency. There is some loss of ascorbic acid in the

preparation of jellies and jams. It is not as great as might be expected, and there is some evidence that the sugar has a stabilizing effect on this vitamin.

Cold has been used for a long time both on a large and a small scale for the preservation of foods. The old-fashioned household icebox, the modern automatic household refrigerator, and the "cold-storage" of eggs, meat, and butter are all commonplace subjects. Low temperatures kill few bacteria but they prevent the growth of most of them. In order to keep foods for long periods of time in cold-storage warehouses, the optimum temperature must be used for each type of food. Meat and poultry are better preserved in the frozen condition. Fish should also be frozen, then dipped in water and refrozen; the coating of ice prevents loss of moisture. Most other foods should not be frozen by ordinary methods but can be kept fairly well in the cold for long periods. However, if the modern "quick-freeze" procedure is used, a great many foods may be kept in the frozen condition indefinitely. When foods are frozen slowly, the ice crystals formed may be many times the size of the individual cells. This causes damage to the cells and, upon thawing, there is a change in texture and leakage of the cell contents and consequent changes in flavor. In quick freezing the usual temperature range is from  $-17.8$  to  $-40^{\circ}\text{C}$ . When foods are rapidly reduced to such temperatures, the ice crystals formed are much smaller and the damage to cell structure is negligible. As much waste as possible is removed before blanching (when necessary) and freezing; the work must be done swiftly to avoid any decomposition, and packaging must be as moisture-proof and air-proof as possible to prevent drying and the action of the oxidizing enzymes, respectively. Quick-frozen or "frosted" foods include fruits and vegetables and meat, fish, and poultry. The process seems to lessen cooking time and to make meats more tender. Microorganisms cannot develop at this low temperature, and all nutritive constituents, including vitamins, are preserved. However, such foods must be kept cold until just before they are ready for cooking, because they rapidly lose their ascorbic acid content after thawing. This can be avoided by cooking without defrosting. Thiamine is not affected by the freezing process, but considerable loss may occur in the blanching of vegetables prior to freezing. In general, however, the nutritive value of frosted foods compares very favorably with that of fresh or canned foods.

Drying foods likewise has both an old and a new aspect. Meats and fish have been sun-dried for centuries, and the same is true of fruits and vegetables. This preserves foods quite well if the drying is quickly accomplished. Consequently artificial driers have been devised which are an improvement on sun and wind. Usually the flavor suffers more than the food value, but the vitamin A and C values diminish considerably unless great care is exercised to prevent oxidation during the drying process. Dehydration of foods was stimulated by war conditions. The economy of shipping dehydrated foods and the importance of carrying such foods as emergency rations by soldiers was apparent. Some progress was made but the development of "hay" flavor after the dried vegetable has been kept for a few weeks has been difficult to overcome.



The standard dried fruits are prunes, raisins, apples, figs, dates, apricots, and peaches. Dried milk is also a useful product, and dried eggs have been used in the baking industry for many years.

Home canning and commercial canning are still probably the most important methods of food preservation. Commercial canning has reached a very high point from a scientific as well as from an economic consideration. The former dangers from canned foods are well-nigh obsolete. These dangers arose from (1) defective containers, (2) poor foods, and (3) insufficient processing. Today almost the only possibility of a defective container is as a result of damage in transit. A safe rule is to never accept a can of food if the container is bent or bulges, or if the label shows evidence that the contents have leaked out. As regards poor food, the reverse is usually the case. Today the canner insists on high-grade materials, in some cases even furnishing the growers with the seedlings or the seeds of the particular variety of vegetable he wants grown for him. The process of commercial canning is a science in itself. The principle, discovered by Appert, a Frenchman, in 1804, is that foods, heated in a container and sealed in such a way that no air is left inside, will keep indefinitely if heated again for a sufficient length of time. The temperature and duration of heating varies with different foods. The basic operations in canning are: (1) cleansing, (2) blanching, i.e., application of warm or hot water, or steam, (3) filling into cans, (4) heating and exhausting air and other gases, (5) sealing the tin-plated container, and (6) heat-processing the sealed container.

The blanch softens the fibrous plant tissue and inhibits the enzymes which might tend to digest or spoil the food. However, it is also the cause of loss of some carbohydrate, minerals, and vitamins. In general, however, the canning process *per se* tends to retain the vitamin content of most foods, except beans and peas, in which losses of ascorbic acid, thiamine, riboflavin, and niacin occur. During storage of canned goods, however, a gradual deterioration of vitamin values usually occurs. This can be lessened by storage at low temperatures. (Guerrant; Sheft.) Different foods vary in this respect. It is therefore evident that the housewife should not store canned foods for long periods of time.

### Food Allergy

Many individuals are peculiarly sensitive to certain foods, just as others are to pollen or other particles in the air they breathe. The symptoms range from sneezing to vomiting, from headaches to hives, from edema to diarrhea and many more, some minor and some quite serious. These effects are believed to be due to the release of histamine by an immunological reaction. The same symptom, or group of symptoms, usually occurs in a given individual, but they may be caused by more than one food. Proteins have been considered the causative agents, and undoubtedly are in most cases, but there are some assertions in the literature that fats and even carbohydrates have been found responsible. The discovery of the "allergen" in any particular case is often not an easy matter. One method consists of injecting the isolated concentrated proteins from different foods into the superficial layers of the skin or applying the protein to a scratch made in the skin. A number of these are done by the physician



at one session. A positive reaction is indicated by a wheal or "hive" around the applied protein. Sometimes the results are inconclusive or negative. In that event the patient is put on successive standard elimination diets. These are standardized diets, each one eliminating certain foods or food groups. If the patient is without symptoms on one of these, it indicates that he is not sensitive to the constituents of that diet. Then foods which are absent from the basic diet are added one at a time until a reaction, peculiar to the patient, is produced by one or more of them. The most usual allergens are milk, eggs, wheat, and potatoes. In conducting such an investigation it may be necessary to add vitamin concentrates since the elimination diets may very well be low in one or another of the vitamins. The best treatment, after the offending food or foods is determined, is to plan an adequate diet which will not contain the allergens.

### Diet Therapy

For specific directions, diet lists, recipes, etc., in handling diets in disease, reference must be made to works on nutrition, dietotherapy, and medicine. Here, only the biochemical aspects will be outlined and, in general, only the relationship of foods—not other types of treatment—will be considered.

**Obesity.**—Obesity is caused by an oversupply of calories, which may be due to overeating, to underexercising, or to a combination of both. The possible influence of endocrine dysfunction will be discussed in another chapter. From a dietary standpoint, a reduction in weight may be achieved by providing fewer calories than are needed by the individual, irrespective of their source or balance. This obviously is an unsound method, although many of the popular diet systems of reducing are based on some such one-sided diet. The physiological method is to ingest a diet which has a normal balance, with an adequate amount of protein and all the essential mineral and vitamin factors. The caloric requirement of the individual should be calculated on two bases: (1) on his or her present weight and (2) on the standard weight for his height and age. Unless the weight reduction desired is not very great, it is unwise to plan the diet immediately on the basis of the desired lower weight. Very rapid reduction is unwise because it involves the quick consumption of the body fat stores and is therefore equivalent to a predominantly fat diet, which often produces ketosis. Consequently when the weight is reduced at a rapid rate there frequently results acidosis, with accompanying unpleasant symptoms. A safe rule is to reduce about a pound a week. Since body fat is about one-fifth water, a pound is equivalent to  $454 \text{ grams} \times \frac{4}{5} \times 9 \text{ Cal./Gm.}$ , or about 3,200 calories. In other words, the maintenance diet for the present weight may be reduced by about 3,200 Cal. per week, or from 450 to 500 Cal. per day. This should be taken out of the fat allowance primarily and carbohydrates secondarily. If reduction does not immediately occur, the diet need not necessarily be changed, because frequently water is held temporarily in the tissues in place of fat and is lost later on. That is, the actual fat has been utilized but this has not become apparent at once. Barach recommends that two-thirds of the required calories be derived from carbohydrates and one-sixth each from proteins and fats.

Exercise will contribute little to weight reduction. It is advised, in moderation of course, in order to improve physical well-being and muscular tone.

**Malnutrition.**—Underweight is frequently more difficult to combat than obesity. If no definite pathology is apparent to account for the condition, dietary measures should be advised. A good multivitamin supplement is indicated to take care of any unsuspected deficiency and to stimulate appetite. High fat and carbohydrate foods of rather concentrated types are recommended if the patient can digest them. An adequate protein intake of varied nature should be provided, but an excess is to be avoided. This is because protein has a higher specific dynamic action than carbohydrates and fats. That is, proteins stimulate the metabolic processes of the body in a peculiar manner. (See page 570.)

**Gastrointestinal Disorders.**—Hyperchlorhydria is a symptom which is frequently encountered. It must be remembered that HCl is secreted at a constant concentration; hence, high acidity simply means an increased volume of gastric juice, too large to be buffered in the normal manner. One method of combating it is to eliminate highly spiced foods from the dietary since these stimulate the flow of gastric juice. Another way is to feed a high protein diet since the proteins combine with HCl to form a relatively weak acid. The reverse condition, hypoacidity, is treated by giving stimulating, but easily digested, foods with relatively low protein and fat content. In gastritis dietary treatment is usually a bland, smooth, soft diet, the composition of which will depend upon whether hypo- or hyperacidity is present. In peptic ulcer, hyperacidity is usually present as well as high pepsin values. This condition is very common and very serious. Many special diets have been devised and recommended. In general, peptic ulcer diets consist of frequent feedings of small quantities of liquid foods which constantly neutralize the secreted acid and prevent its accumulation as free acid, but the amount and kind of food must be regulated to satisfy all the nutritional requirements. Co Tui and co-workers have recommended, in the management of gastrointestinal ulcers, the feeding of protein hydrolysates in large amounts, reinforced with high caloric supplements. The explanation for the favorable effect of this "hyperalimentation regimen" is that the amino acids buffer the HCl and also enable the body to repair the damage to its protein. At the same time the digestive effort of the gastrointestinal canal is lessened.

Flatulence or gas formation in the gastrointestinal canal occurs both in health and in disease. It is most distressing after an abdominal operation, but in such a case it does not appear to have much relation to foods but to a lack of intestinal tonus or inhibition of peristalsis followed by diffusion of blood gases into the intestine. In general, a lack of HCl in the stomach may permit more microorganisms than usual to pass into the duodenum, and gas formation ensues as a result of their growth. Some foods are more productive of gas than others. Well-known examples are members of the cabbage family and also turnips, onions, peas, and beans. Melons, cucumbers, and radishes are other offenders in this respect. Various syrups also are gas producers, perhaps serving as media for yeasts in the lower part of the tract.



**Diseases of the Liver and Gall Bladder.**—In liver diseases a high carbohydrate, moderately high protein, and moderately low fat diet is indicated. Liver cells are much more readily restored to normal function if their glycogen content is built up. A protein intake of from 70 to 100 grams is recommended for several reasons: (1) it promotes repair of damaged cells, (2) it appears to have a protective function, and (3) it tends to overcome hypoproteinemia which may occur in hepatic conditions. The load of protein should not be too great because the liver is the site of protein degradation and formation of urea, and this might produce too much of a strain on its powers. The low fat is recommended because fat requires bile secretion for its digestion and absorption, and this hepatic function must be spared as much as possible. Since bile secretion is depressed, the fat-soluble vitamins will not be absorbed in sufficient amounts. For this reason, vitamin supplements are advised—in some instances even administered parenterally. Indeed, if the patient is unable to eat, intravenous feeding of amino acids, together with glucose, fats, vitamins, and salts, may be instituted. In gall bladder conditions, dietary treatment depends largely on whether blood cholesterol is high or not. If it is high and is associated with gallstones, foods rich in cholesterol are excluded from the diet. (See Table XX.) Other fatty foods, such as olive oil, may be given to stimulate the emptying of the gall bladder. High protein also has this action. If there is no hypercholesterolemia, the avoidance of cholesterol in the diet is not necessary, and a high fat diet may be helpful by stimulating the gall bladder to contract.

**Celiac Disease.**—Celiac disease has as its principal symptom fatty stools (steatorrhea). It occurs chiefly in children. The abdomen is greatly distended, largely because of accumulation of intestinal gas. At the same time there is a complete metabolic upset; fat stores are used up and growth is stunted. Similar conditions are nontropical sprue of adults and tropical sprue. Fatty stools are common to all three conditions, with a consequent loss of calcium and vitamin D via the feces. Besides an inability to digest and absorb fats, there is frequently also an interference with starch digestion. The first step in diet therapy is to give a high protein diet, next add fruits, and then a small amount of easily digested starchy food. Bananas are particularly well tolerated in these conditions. Finally, one egg yolk a day is permitted because of its fat-soluble vitamin and lecithin content. Further fat additions are inadvisable.

A number of different dietary regimes have been recommended for these conditions. Recent European studies suggest that wheat flour is to be avoided in celiac disease. One of its proteins, gliadin, seems to be harmful. The explanation for the beneficial effects of bananas and other fruits lies in the diminution in the amount of wheat products ingested. It is also maintained that unsaturated oils, such as olive and soybean oils, are better tolerated in celiac disease.

**Kidney Conditions.**—The diet in acute glomerulonephritis is planned to relieve the kidney without regard to the total nutritional needs of the patient because of the short period of time involved. A low protein diet with little of anything else is given in order that a urine low in total solids will be excreted by the abnormal kidney. The total fluid intake should be adequate



to dissolve the urinary constituents easily but not too great to put a heavy load on the kidney—perhaps from 1,000 to 1,200 c.c. daily. As soon as the condition is corrected the protein intake is restored to normal. In chronic glomerulonephritis there is a failure to secrete the nonprotein waste constituents which results in accumulation of these constituents in the blood, and also a loss of serum protein in the urine. In the past, both of these phenomena influenced the physician to restrict protein in the diet—in the first instance because more protein led to a greater production of urea, uric acid, etc., for the kidney to eliminate, and in the second instance because it was felt that the feeding of protein led to a greater excretion of it by the kidney. At present it is considered more important to make good the loss of protein from the blood by feeding extra protein. Furthermore, a low serum protein usually occurs or threatens to occur, and a high protein diet tends to counteract this. Consequently the general trend is to feed the normal requirement of protein plus an amount equal to that lost by way of the urine. Some clinicians have favored restricting the salt intake drastically, but today this is not generally accepted. A moderate restriction, say to 2 Gm. per day, is often advised. It also seems wise to have the diet tend more to the basic ash type because acidic constituents are less easily excreted by the kidney. The total water intake should be nearer 1 than 2 liters. In nephrosis, or degenerative Bright's disease, the same principle regarding protein is applied. The condition called "lipoid nephrosis" may be a metabolic disturbance rather than a kidney disease. Edema, oliguria (small volume of urine), marked proteinuria and low serum proteins occur. There is no retention of nitrogenous products in the blood. Very high protein diets, containing from 120 to 240 Gm. of protein per day, with low carbohydrate and very low fat have been widely used (Epstein). In conditions in which the proteinuria is not pronounced (nephrosclerosis), a balanced diet with moderately high protein is recommended.

**Urinary Calculi.**—Calculi which form in the kidney or urinary bladder are usually mainly composed of urates, oxalates, or phosphates. Their formation will be considered when urinary constituents are discussed. From the dietary standpoint it should be pointed out that urates arise from nucleoprotein metabolism. Therefore, persons who have had urate stones should be on a low nucleoprotein diet since urinary calculi are very likely to recur. Glandular meats are richest in nucleoproteins, but nonglandular meats are relatively high as are also the germ of grains and the actively growing parts of plants, such as the tips of asparagus, soybean sprouts, etc. Anchovies, sardines, and caviar also contain considerable quantities of nucleoproteins. The reason for greater oxalate excretion in the urine by some individuals than by others is not well understood. Undoubtedly some oxalate originates in metabolism. However, until our knowledge of the underlying reactions is better known, the only dietary suggestion which seems logical is to avoid foods having a high preformed oxalate content. These include spinach, chard, beets, beet leaves, rhubarb, tomatoes, figs, okra, gooseberries, sweet potatoes, cocoa, chocolate, and tea. Phosphates precipitate in an alkaline urine. Therefore, the indicated diet for phosphaturia would be an acid-ash diet low in phosphorus. Phospho-

proteins, nucleoproteins, and phospholipids all contain phosphorus. Hence, milk, glandular foods, and eggs should be given in moderation, and meat and cereals, both of which are acid forming, in increased amounts.

**Gout.**—Clinicians are not in agreement regarding the underlying cause of gout. It has been stated generally that purine metabolism is not deranged but that some fault in the excretion exists—that before and during an acute attack the excretion by the kidney is halted. Talbot and Coombs maintain that this condition exists only in elderly patients whose kidneys have been damaged. In patients with normal kidneys there is, in fact, an increased excretion of uric acid. They correlate the attacks with an increase in weight, due to a diminution in insensible perspiration, which leads to a diuresis immediately before or during the actual attack. Although foods with a high uric acid content should be avoided, no radical change in diet produces any marked amelioration, according to these observers. Conservative dietary precautions, based on the older theories, call for a diminution of total caloric intake of from 10 to 15 per cent, with low protein and low fat, to reduce the patient's weight and keep it reduced, a purine-free diet, and a long continuance of this regimen.

**Circulatory Disturbances.**—Dietary measures may be of considerable value in disorders of the heart and blood vessels. Obesity is a major obstacle in treating heart trouble. According to McLester, the disadvantages of obesity may be fourfold. First, even when the heart is unimpaired, a large body mass puts an abnormally great strain on the heart. Second, in obesity there frequently occur fat deposits upon and between the cardiac muscle bundles decreasing the heart muscle efficiency. Third, abdominal fat may impede the movement of the diaphragm, and this in turn affects the heart movements. Finally, arteriosclerosis, which often accompanies obesity, may invade coronary vessels and in that way directly affect the blood supply of the heart. Accordingly, cardiac patients are usually advised to reduce their weight to normal or slightly below. As in all reducing regimens, this must not occur too quickly. A plentiful supply of vitamins, particularly the B complex and C, should be assured and the protein should be adequate. In arteriosclerosis and hypertension, much the same dietary advice is given, stressing moderation and, perhaps, mild undernutrition.

The relationship of cholesterol to atherosclerosis, a form of arteriosclerosis, is discussed on page 467. Some physicians advise low cholesterol diets in this condition, while others believe such diets are of little value unless the cholesterol content can be brought to an extremely low value. In familial hypercholesteremia, however, low cholesterol diets are useful. In Table XXX are given the cholesterol concentrations of a number of typical foods. These are all of animal origin; plants contain no cholesterol.

**Hypertension.**—Despite the general opinion to the contrary, protein foods do not cause an elevation of blood pressure. Consequently they do not need to be reduced in the diet in hypertension unless there is serious kidney impairment at the same time. The preference for white meats over red meats also seems to be without foundation. The question of salt restriction in hyper-



TABLE XXX  
CHOLESTEROL CONTENT OF FOODS\*

FOOD	CHOLESTEROL (MG. PER 100 GM. FRESH MATERIAL)	FOOD	CHOLESTEROL (MG. PER 100 GM. FRESH MATERIAL)
Bacon	38-78	Fish	21-95
Beef	38-78	Kidney	20-3,400
Brain	2,130-3,700	Liver	130-3,400
Butter	185-340	Milk, cow's, whole	12
Cheese	42-88	Milk, cow's, skim	2
Chicken	59-527	Oysters	215
Egg, whole	240-490	Pancreas	3,120
Egg, yolk	1,180-2,150	Pork	46-48
Fats (lard, suet)	100-350	Veal	84-88

\*Data from Twiss, J. R., and Greene, C. H.: *J. A. M. A.* **101**: 1841, 1933; Cook, R. P.: *Nutrition Abstr. & Rev.* **12**: 1, 1942; Okey, R.: *J. Am. Dietet. A.* **21**: 341, 1945; Mann, G. V.: *J. Am. Dietet. A.* **25**: 389, 1949.

tension is controversial, but there seems to be increasing evidence that a drastic reduction in sodium intake is beneficial (Chapman and Gibbons). The so-called "rice diet" therapy has been proposed by Kempner, who finds a favorable influence in a fair percentage of cases of hypertension. The diet consists of rice, fruit, and sugar, with little else, except vitamin supplements, and essentially is a low protein, low salt regime. It would appear dangerous to restrict proteins drastically for too long a time. A more palatable and acceptable diet is recommended by Ornstein. By a judicious selection of foods low in sodium (see Table LIII), and by removal of salts by water extraction and by dialysis, a diet extremely low in sodium, but not low in proteins, is achieved. Water-soluble vitamins must be added. The results have been very favorable.

**Diabetes Mellitus.**—For our present purposes we may say that this is a condition in which carbohydrate, arising from whatever source, is not utilized by the body. As a result, the glucose of the blood rises and may "spill over" into the urine. Treatment consists in diet alone, or diet plus insulin, the hormone which has a regulatory effect upon the level of blood sugar. If a diet can be arranged which will keep the blood sugar at or near the normal level and will at the same time provide sufficient calories and protein, no insulin is needed. The amount of carbohydrate which 1 "unit" of insulin will aid the body to handle varies with the individual, but it is about 1 to 3 Gm. There are various systems of adjusting diabetic diets. The Sansum diet is high carbohydrate, low protein, and low fat; the Newburg diet is high fat and low carbohydrate. Joslin advocates protein at a normal level, carbohydrate somewhat low, and fat moderately high. In planning these diets the patient must be taught the use of food tables, how to weigh his diet, and the calculations involved. Many patients administer insulin to themselves and become adept in managing their therapeutic regime.

**Addison's Disease.**—Optimal therapeutic results in the treatment of Addison's disease require not only the administration of large amounts of sodium salts and the adrenal cortical hormones, but also the restriction of the intake of potassium. Since potassium is widely distributed and is especially high in protein foods, which the patient needs in large amounts, the problem is a very



difficult one. The extraction of potassium from food is likely to result in a tasteless meal which the patient will not eat. Vegetables should be specially cooked in salt ( $\text{NaCl}$ ) water. The  $\text{NaCl}$  will diffuse into the vegetables and the potassium salts will go out into the cook water because the latter has a low potassium concentration. This fluid is then discarded. Since the water-soluble vitamins are lost along with the potassium salts, provision must be made, of course, for the addition of the appropriate vitamin supplements. A similar procedure is used for meats, which are first placed in parchment bags. By these methods the potassium content is reduced to one-third or one-fourth its original concentration. Foods to be avoided are soups and broths containing meat stock or meat extracts, gravies (except those prepared by a special method), catsup, chili sauce, mustard, dried fruits and vegetables, nuts, peanut butter, molasses, caviar, chocolate, cocoa, postum, and spinach. (Victor.)

**Epilepsy.**—In recent years a ketogenic diet has been advised in the treatment of epilepsy, because it was found that an acidosis tends to lessen the number and the severity of the seizures. The ketone bodies arise when the fatty acids are incompletely oxidized, and when this occurs to an excessive degree acidosis of more or less severity results. (See Chapter 17.) A ketogenic diet is one high in fats and low in carbohydrates. The protein requirement is said to be higher than normal on such a diet and 2 Gm. of protein per kilogram of body weight are recommended. Since the tendency to retain water is regarded as one of the factors in producing seizures in epilepsy, the total water intake should be somewhat restricted and for the same reason the salt in the food should be held at a low level. Even with a high quota of protein, the ketogenic diet is unpalatable and difficult to take. The large amounts of fat may be nauseating if the full diet is suddenly given. It is therefore best to change the diet gradually and, once it is in effect, it should be tried for a long time before good results are to be expected. (Keith.)

**Fevers.**—In fevers metabolism is increased. DuBois calculates the total caloric requirements in fever by adding 13 per cent of the normal basal metabolic rate for the individual for every degree of fever. To this is added an additional 10 per cent if there is much extra protein catabolism, as there usually is in most fevers, and from 10 to 30 per cent for the restlessness of the patient. It is thus seen that the caloric needs of a febrile patient may be very high indeed. The diet therefore should be high in carbohydrates to provide for much of this metabolic need, to spare proteins so far as possible, and to aid in combating acidosis. Proteins should be sufficiently high to maintain the patient in nitrogen equilibrium. Fats should be normal in quantity and of a type easily digested. In fevers an alkaline-ash diet is preferred as a further safeguard against acidosis, and additions of sodium chloride to the diet will make good the losses of salt in perspiration.

**Preoperative Diets.**—A good nutritive condition is a great asset before an operation. If an operation can be anticipated the surgeon will exercise every care to build up his patient. If, just prior to an operation, the diet is restricted in bulk, it will serve the same purpose as the old-fashioned purge which both weakened the patient and drained off some of his much needed

body water. Fluids and carbohydrates, sugar candy for example, are given in fairly large amounts on the preceding day and no food after the evening meal if the operation is in the morning, because an empty stomach at the time of operation is essential. Postoperative diets will depend upon the type of operation, condition of the patient, etc.

**Parenteral Feeding.**—The intravenous administration of amino acids is discussed on page 405 in some detail. Carbohydrates, vitamins, and salts can also be given intravenously, but an adequate caloric ration cannot be supplied from the amount of amino acids and carbohydrates which it is possible to inject. The substitution of invert sugar for glucose is said to be advantageous. Twice as much can be administered per hour, probably because of the more rapid utilization of the fructose portion or because of a catalytic influence of the fructose on the glucose portion. (Weichselbaum; Corkill and Nelson.) Nevertheless, it is evident that fats must be included because of their high caloric value, and recently they have been used safely after numerous animal experiments. The preparation of a suitable emulsion has been a difficult problem. Various stabilizers have been employed to maintain extremely fine emulsions for hypodermoclysis, as well as for intravenous administration. (Waddell; Shafiroff.) Fat, labeled with radioactive carbon atoms and emulsified, had previously been shown to be converted into  $C^{14}O_2$  after intravenous injection into rats. The  $C^{14}O_2$  was discovered in the expired air. This indicated that not only is intravenously administered fat safe, but it is definitely utilized. (Geyer; Lerner.) It is probable that mixtures of amino acids, glucose, etc., with such emulsions will eventually provide balanced intravenous "meals" for persons unable to be fed orally.

### Unbalanced and Incomplete Diets

Too much emphasis cannot be placed on the possibility of improper diets being chosen and administered for long periods of time, because only the patient's predominant symptom or ailment is considered. This has been mentioned several times in various connections. It should be remembered that in liquid diets a few foodstuffs are likely to predominate. If such a diet is continued unduly, the patient is likely to ingest an unbalanced supply of amino acids and convalescence would thereby be prolonged. Consequently, care should be taken to vary the protein constituents. Monotonous, one-sided diets are sometimes seen in poorly managed institutions for orphans, the mentally deranged, or prisons. Other practices lead to nutritional upsets. Among these are the use of large volume of saline infusions with a tendency to wash out the body's supply of water-soluble vitamins and the administration of excessive amounts of mineral oil leading to loss of fat-soluble vitamins.

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## Chapter 14

### PHYSIOLOGICAL OXIDATIONS

General or external respiration involves the inspiration of oxygen from the air and its transportation by the blood stream to the tissues. It further includes the various mechanisms which permit large amounts of carbon dioxide to be carried to the lungs where it is eliminated (Chapter 20). In the tissue capillaries oxyhemoglobin dissociates, transferring a portion of its oxygen to receptors present in the tissue juices and in the cells. The actual utilization of oxygen and the production of carbon dioxide and water then take place in the reverse processes of cellular or internal respiration. In the course of these reactions there occur energy exchanges, which are just as vital as the purely chemical reactions of oxidation and reduction. Both types form the subject of the present chapter. They are introduced here because the reactions described are involved in the intermediary metabolism of proteins, carbohydrates, and lipids, which are considered in subsequent chapters.

#### OXIDATIONS

The term "oxidation" is applied to protoplasmic oxidations in the same senses as in nonvital oxidations—i.e., combination with oxygen or removal of hydrogen—in any case, a loss of electrons. In some of the complex reactions the transfer of electrons may be difficult to express, particularly in our present state of knowledge. It is perhaps unnecessary to remark that every oxidation must be accompanied by a reduction. It might also be pertinent to note that, as Gerard has pointed out, oxidation in itself cannot be a source of energy. Oxidation involves an increase in valence, i.e., a removal of electrons, which requires energy. It is the concomitant reduction that yields energy because with reduction there is an addition of electrons. However, since every oxidation is accompanied by a reduction, it amounts to the same thing and it also may be stated that oxygen ranks high as a hydrogen acceptor.

Now, although not all oxidations involve oxygen, nevertheless oxygen is a vital requirement. Anaerobic organisms may and do perform oxidative reactions in the absence of oxygen and even aerobic organisms are able to do so under some circumstances. However, a human being cannot live in an oxygen-free atmosphere for more than about three minutes. It is probable that most, if not all, human tissues require oxygen for the completion of their vital oxidations, even though the intermediate stages may go on in its absence.

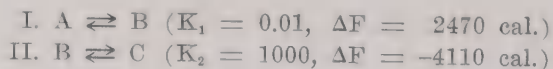
The reactions taking place in cellular respiration are known as "physiological oxidations." It must be remembered that molecular oxygen is incapable of oxidizing physiological substances outside of the body at body temperature except to a very slight extent. Within the body such oxidations are

occurring constantly. It is therefore evident that the body must possess means for making such reactions possible at such low temperatures. The two problems before us are (1) how the cell brings about oxidation of its substrates at the low temperature of the body and (2) how the energy derived from the oxidation of this substrate is utilized before being dissipated as heat. These are complicated reactions, many of them "chain reactions," of such a nature that the oxidations are controlled. In this way the energy evolved may be used physiologically.

### Energy Relationships

The degradation of biological substrates in the living cell by the process of physiological oxidation involves changes in which compounds of higher levels of energy go to products having lower levels of energy. This difference between the energy levels of the substrates and their products is termed the free energy of the reaction and is indicated by the symbol  $\Delta F$ . Reactions that yield energy are termed "exergonic" and are thermodynamically spontaneous. Such reactions are generalized under the term "catabolism." However, a reaction, which may be thermodynamically spontaneous, may not occur by itself at any appreciable rate. There is the so-called energy of activation, which is interposed between the initial and final states. It is the catalytic intervention of enzymes that makes possible the multitude of reactions at the rates which are seen in biological systems.

Chemical reactions that require the addition of energy for their occurrence are termed "endergonic" and are representative of anabolic reactions. It should be kept in mind that the catabolism, or breaking down, of physiological substrates has as its prime purpose the maintenance of anabolic reactions, as typified in growth and in the performance of biological work. Such endergonic reactions must, of course, be driven by the utilization of part of the energy of an exergonic reaction. The only mechanism available for such a transfer of chemical bond energy from one reaction to another is by the utilization of *a common reactant of both reactions*. Consider Reactions I and II where  $K$  = the equilibrium constant and  $\Delta F$  = the free energy change between the initial compound and its product:



Since Reaction I is endergonic, it will proceed to the left unless the concentration of B is less than 1 per cent of the concentration of A. However, since Reaction II is exergonic, it will proceed to the right until the concentration of B is less than 0.1 per cent of C. Thus, B will be removed from the coupled reactions as fast as it is formed from Reaction I. Consequently it becomes possible for Reaction I to proceed to the right. Energetically, the over-all reaction  $A \rightleftharpoons C$  may be considered in terms of the  $K$  values and the  $\Delta F$  values; that is,

$$\begin{array}{l} K = K_1 K_2 = (0.01)(1000) = 10 \\ \text{and } \Delta F = (2470 - 4110) = -1640 \text{ cal.} \end{array}$$



Thus the reaction proceeds to the right until the concentration of C is ten times that of A, and it is a net exergonic reaction. Another principle, demonstrated in this typical example, is the relationship between the endergonic, or driven, reaction and the exergonic, or driving, reaction. The driven reaction must precede the driving reaction.

In biological systems, phosphate compounds occupy the unique position of being the common reactant in a multitude of reactions. Most particularly is this function seen in adenosine triphosphate and other high energy compounds. These will be discussed later in this chapter.

**Terminology.**—The biochemical agents involved are *enzymes*, *coenzymes*, and *hydrogen acceptors* or *carriers*. Those enzymes which act on the substrate and make possible the removal of hydrogen from it are called dehydrogenases, and those which act on oxygen and cause it to take part in an oxidative chain are termed oxidases. These enzymes are rather specific; there are many dehydrogenases and a number of oxidases. Hydrogen carriers or acceptors are defined as compounds which, by virtue of their ability to be oxidized and reduced, function in the transport of hydrogens or electrons from tissue metabolites to oxygen or some other oxidizer. In general they are also of a complex protein nature but are characterized by particular prosthetic or active groups which make possible their specific functions. Certain coenzymes, namely, coenzyme I and coenzyme II, which will be discussed more fully later, are dissociable from their protein enzyme fractions but cannot function independently as carriers.

**Methods of Study.**—Since the respiration of tissues involves the utilization of oxygen, either immediately or eventually, it is natural that the primary method of evaluation of such activity should involve a direct measurement of oxygen uptake. The development by Warburg of a microrespirometer provided the basic tool for investigation in this field. While many variations of this instrument have been devised, the techniques originally described by him are still widely used. Fig. 43 is a diagram of this instrument.

In general, measurements may be made on three main types of test materials: (1) tissue slices, (2) tissue homogenates (fine minces), and (3) isolated components of the enzyme systems to be studied. Certain intrinsic limitations exist in each of these test materials. The purer the chemical system under study, the less one may infer as to the direct participation of the system in intact tissues, while the results obtained with tissue slices often fail to disclose the complexity of the enzyme systems involved, since one sees only the end results.

Investigation of the dehydrogenase activity of tissue slices, homogenates, or isolated enzyme systems has also been accomplished by means of the Thunberg technique. Here measurements are made of the rate of anaerobic decolorization of methylene blue. The methylene blue functions as a hydrogen acceptor, and the velocity of its reduction is thus a measure of the activity of the respiratory enzymes.

More recently, tetrazolium salts have been employed to measure dehydrogenase activity of tissue slices and homogenates. The tetrazolium salts are water soluble and essentially colorless. By means of dehydrogenase or flavo-

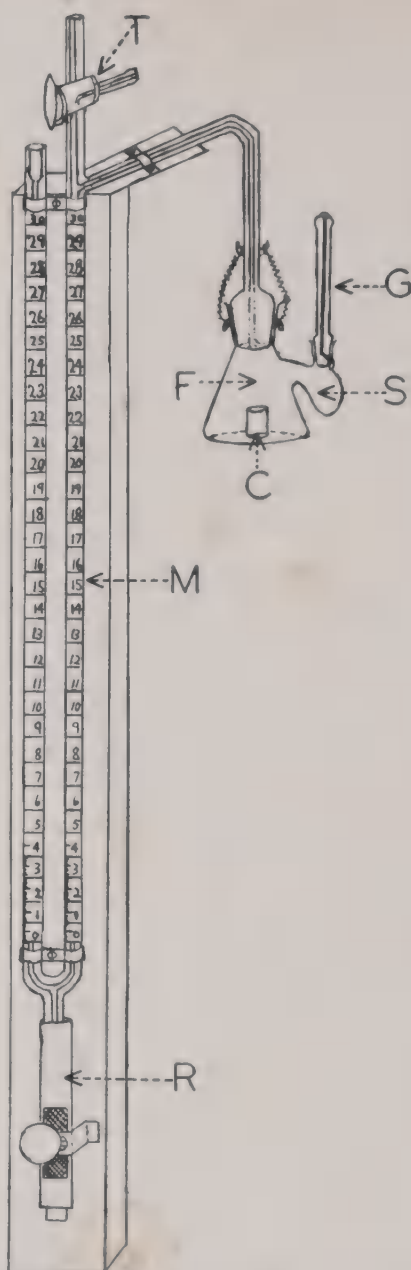


Fig. 43.—The Warburg constant volume manometer. *F*, Flask; *S*, sidearm; *G*, sidearm stopper with gas vent; *C*, center well (for alkali); *M*, manometer proper containing a liquid of known density; *R*, fluid reservoir, which, by adjustment of the screw clamp, permits of alteration of the level of the fluid in the manometer; *T*, three-way stopcock. The scale of the manometer is graduated in centimeters and millimeters.

The principle involved is that, if the volume of a gas is held constant at constant temperature, any changes in the amount of gas can be measured by measuring changes in the pressure. In this apparatus, for example, if it is desired to determine the oxygen uptake of tissue, the tissue is placed in the flask *F*. The sidearm *S* is provided so that a given reagent can be added during the course of the experiment. The flask has a center well, *C*, which contains alkali; this absorbs any  $\text{CO}_2$  formed during the experiment. After introducing the tissue flask is immersed in a water bath at constant temperature. The entire system is filled with oxygen, and the intervals, and between readings the system is shaken to promote a rapid gas exchange between the fluid and the gas phase. As oxygen is absorbed by the tissue, the level of the fluid in the manometer will change and the amount can be determined by suitable calculations. (From Umbreit, W. W., Burris, R. H., and Stauffer, J. F.: *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, Minneapolis, Minn., 1945, Burgess Publishing Co.)

protein activity, they are reduced to colored water-insoluble formazans. The intensity of such enzymatic activity may be determined by the extraction and colorimetric measurement of the formazan, giving one the amount of tetrazolium reduced per milligram of tissue. This procedure also allows of a unique visualization of the intracellular localization of such activity by microscopic examination of frozen sections of the tissues. (Black and Kleiner.)

**Cellular Localization of Enzyme Activity.**—Investigations in this field have employed histochemical and ultracentrifugation techniques. The former techniques demonstrate a cytoplasmic localization for cytochrome oxidase, peroxidase, and dehydrogenases. Further, in many instances the activity can be seen to be associated with the large granular or mitochondrial fractions of the cell. These findings are confirmed by the study of isolated cellular components obtained after centrifugation. In addition, it has been shown that the enzymes concerned with glycolysis are not associated with particulate fractions and reside largely in the soluble cytoplasmic fraction. The particulate fraction is also the site of the enzymes of pyruvate and fatty acid oxidation as well as of those concerned with phosphate esterification during biological oxidation.

The mitochondria, sometimes termed the large granular fraction, appear to be the major site of activity involved in the terminal stage of electron transport and oxygen activation. In appreciation of their importance in cellular metabolism, Claude has described the mitochondria as intracellular power plants. From the standpoint of chemical composition and structure, the mitochondria are asymmetric structures which contain about 25 per cent of the deoxyribonucleic acid of the cell, in the case of the liver. They show osmotic activity but are not simply small vacuolar osmometers since they can be shown to possess an internal granular structure on electron microscopy. They also contain a considerable amount of phospholipid. It should also be noted that factors of intracellular tonicity may influence enzymatic activity associated with the mitochondria. Thus the functional integrity of various enzyme systems, particularly the cyclophorase system, is dependent upon the structural integrity of the mitochondria.

It is significant to note that synthetic (anabolic) as well as degradative reactions are also associated with mitochondrial enzymes. This occurrence of synthetic and oxidative enzymes in the same structural unit, where exothermic reactions are required to drive endothermic ones, is a remarkable phenomenon. A striking example of the significance of this organization is found in oxidative phosphorylation, which occurs only in intact mitochondria, and is lost on disruption or isolation of the components of the reaction.

The microsomes also contain phospholipids, proteins, and the highest localized concentration of ribose nucleic acid. However, they do not exhibit any great degree of enzyme localization. The supernatant fluid of ultracentrifugal preparations contains proteins, "soluble" enzymes, and smaller amounts of ribose nucleic acid. The nucleus, so significant in genetic considerations and in chromosomal mechanics, is relatively poor in enzymes, with the possible exception of phosphatase.

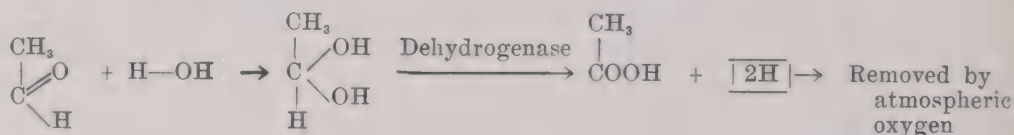


## RESPIRATORY ENZYMES AND CARRIERS

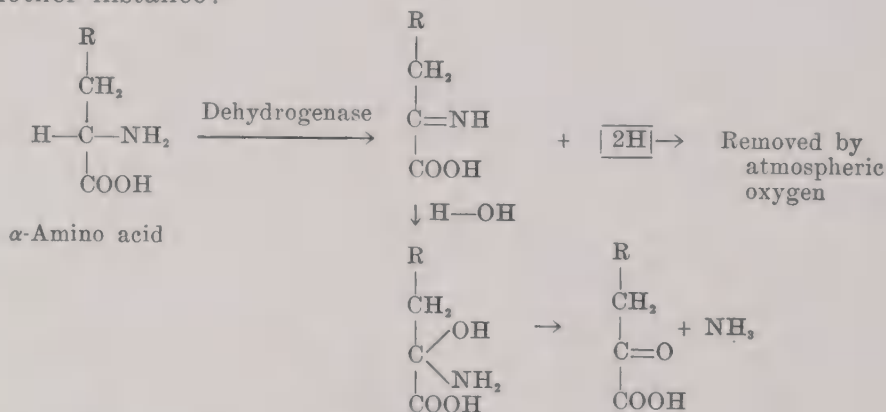
### Hydrogen Activation

We owe the advancement of the hypothesis of hydrogen activation to Wieland. In hydrogen activation, a very common phenomenon, the organic substance to be oxidized is acted upon by a dehydrogenase in such a way that the hydrogen atoms within its molecule become capable of being transferred. It is oxidized by dehydrogenation and the hydrogen removed is simultaneously taken up by a suitable oxidizing agent or even by molecular oxygen. Another conception of dehydrogenation is that the metabolite together with the hydrogen acceptor is adsorbed on the dehydrogenase (protein) molecule in such a way that the hydrogen passes from the metabolite to the hydrogen acceptor. The oxidized metabolite and the reduced carrier then separate from the dehydrogenase and the process can be repeated with another set of metabolite and carrier molecules, the dehydrogenase being used over and over again.

There are many of these dehydrogenases, apparently quite specific as to the substrates from which they remove hydrogen. For example, the oxidation of acetaldehyde to acetic acid may be accomplished in this manner:



Another instance:

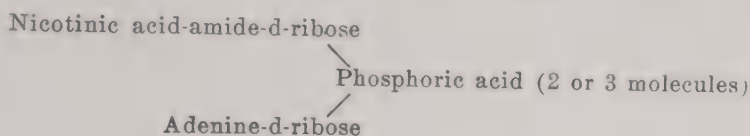


Both of the above are illustrations of oxidations by dehydrogenation. It should also be noted that in both cases there is actually more oxygen in the end products, although neither molecular nor activated oxygen is introduced. Oxygen is eventually required because the hydrogen must at some stage be oxidized to water or to hydrogen peroxide.

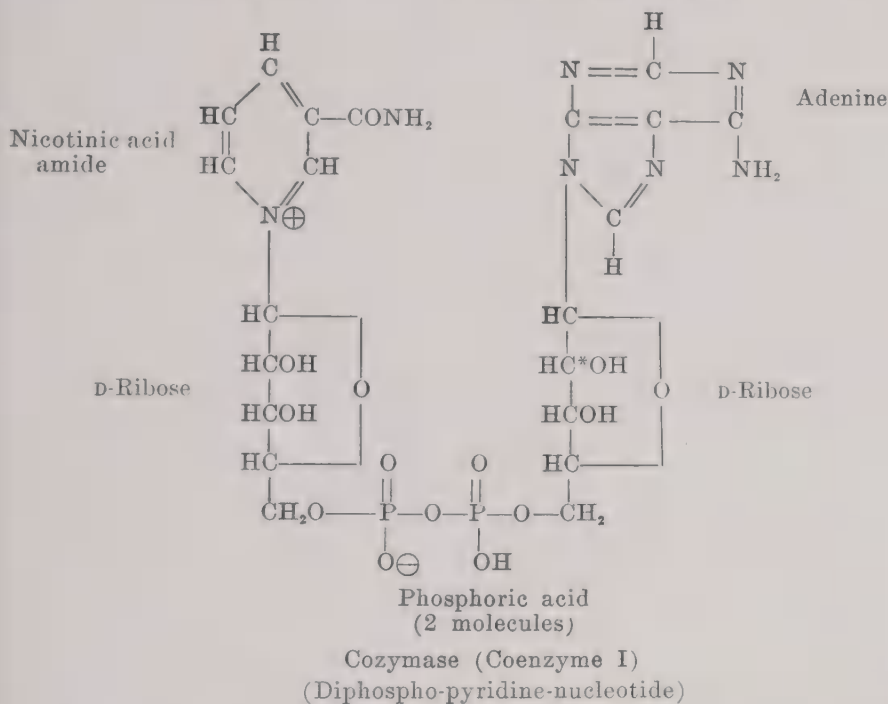
### The Coenzymes

By definition, coenzymes are dialyzable, heat-stable, organic compounds necessary for the functioning of enzymes. In essence they are the free prosthetic groups of the enzymes. The coenzymes of importance in physiological oxidation reactions are the phosphopyridine nucleotides and coenzyme A. The

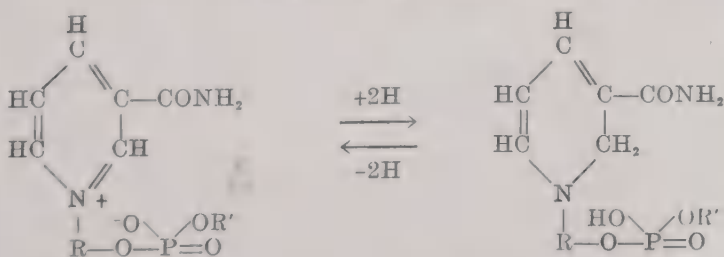
atter will be discussed later. The phosphopyridine nucleotides have the general structure:



Coenzyme I has two phosphoric acid molecules and is known as DPN, or cozymase. Its structural formula is given below. Coenzyme II is the triphosphoric derivative and is often called TPN for triphospho-pyridine-nucleotide. The position of the third phosphoric acid in coenzyme II, as proposed by Kornberg and Wacker, is indicated by the asterisk (\*) in the formula for coenzyme I.



These nucleotides, then, consist of the purine, adenine; 2 molecules of the pentose, d-ribose; 2 or 3 phosphoric acid molecules; and nicotinic acid amide. It is the nicotinic acid amide which imparts to the nucleotide its important reversible oxidation-reduction activity; that is, it is the hydrogen carrier part of this huge molecule. It may be shown in the following way:

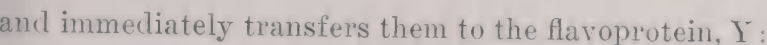


Among the metabolites requiring coenzyme I are the salts of lactic acid, malic acid, and beta hydroxybutyric acid; alcohol; and glyceraldehyde diphosphate; while glucose and glutamates use either coenzyme I or II.





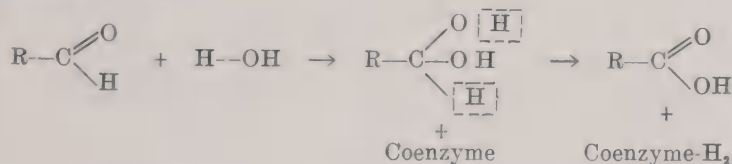
The dehydrogenase activates certain hydrogen atoms of the metabolite and the coenzyme accepts them



For regeneration of the reduced flavoprotein only atmospheric oxygen is necessary:



An example of oxidation by the flavoprotein system is to be found in the oxidation of hexosemonophosphoric acid to phosphohexonic acid. Indicating only the reacting group:



In this system we have two vitamins as essential factors, nicotinic acid amide (niacin) and riboflavin, which are parts of the molecules of the coenzyme and yellow enzyme, respectively.

## The Cytochromes

The third type of oxidation reaction is exemplified by the cytochrome systems. In 1925 Keilin discovered that there were widely distributed in animal tissues certain hemochromogens which he called cytochromes. These combinations of heme and proteins have characteristic absorption spectra differing from those of hemoglobin and its derivatives and are designated as "a," "b," and "c." Cytochrome "c" is the one which has been most carefully studied. It is present in largest amounts and has been isolated in a relatively pure state. The existence of the other cytochromes has been indicated by their characteristic absorption spectra. Chemically cytochrome c is a heme-protein with the heme residue united to the protein by a thio-ether linkage. Characteristic absorption bands are found in the visible spectra at 5507, 5223, and 4150 Å for reduced cytochrome c, while oxidized cytochrome c gives two diffuse bands at about 5300 and 4000 Å.

Cytochrome c's isoelectric point is at pH 9.86, and it contains between 34 and 0.43 per cent of iron. But perhaps most significant from the point of view of the functioning of the oxidation-reduction chain is the fact that from this stage on hydrogen is no longer transported, but instead the changes involve electron transfer. Thus, while the electrons of the substrate are de-

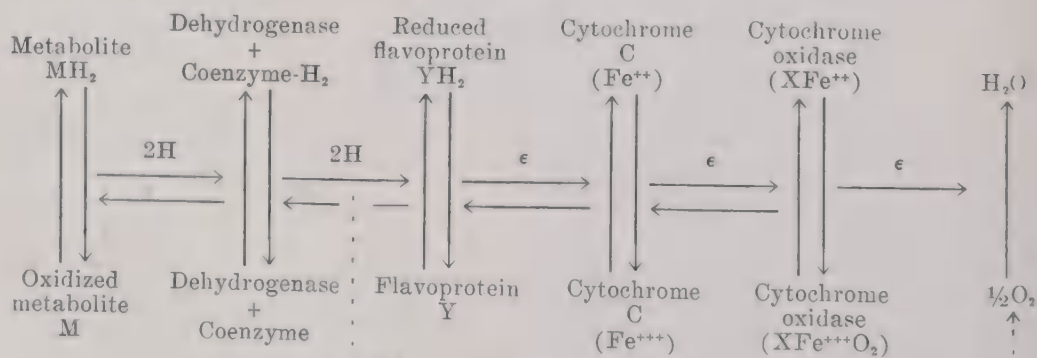
livered to oxygen in a continuous chain, the hydrogen ion may enter or be withdrawn at several places in the chain. Furthermore, the components of the chain may be classified as to their ability to transfer electrons. The cytochromes can transfer but one electron per cycle of oxidation and reduction of their prosthetic group, while the flavoproteins and pyridine nucleotides can transport two electrons per cycle.

Recent work on cytochrome a and cytochrome b indicates that they are mixtures and that at least fractions of b and a are associated with cytochrome c reductase and cytochrome oxidase, respectively. The cytochrome chain would consequently function in the order b-c-a.

A dehydrogenase first activates the hydrogen atoms in a metabolite. These are then accepted or carried by another carrier, such as flavoprotein, to cytochrome. The reduced cytochrome may then be reoxidized by cytochrome oxidase and is ready to accept more electrons.

**Cytochrome Oxidase.**—Cytochrome oxidase has been shown to be identical with "Warburg's respiratory enzyme." It is found in practically all forms of life and the reduced form gives rise to water in its capacity of reducing oxygen so that the latter may combine with hydrogen atoms. It is apparently associated quite firmly with granular constituents of the cytoplasm and thus far has not been obtained in solution. There is reason to believe that it contains an iron atom which can oscillate between the ferrous and ferric state with reduction and oxidation.

The various specialized enzymes and carriers function in a sequential manner in the oxidation of intracellular substrates. An analogy to a "bucket brigade" has been made to show that each reaction is dependent upon all the preceding ones. Dehydrogenation is probably the most common type. The metabolite is first dehydrogenated and the hydrogens or electrons, or both, are passed along from one compound to another. Finally, a system is reached which involves the acceptance of hydrogen by molecular oxygen. Thus, the metabolite is oxidized by dehydrogenation but ultimately oxygen is required for the consummation of this reaction. Several such chains are known and are being actively studied. An example of one such complete hydrogen transport system, in which a number of the reactions already described will be recognized, is shown as follows:



Schematic representation of the hydrogen transport mechanism for metabolites which require coenzyme I or coenzyme II. The horizontal arrows represent the path of hydrogen or electrons. All reactions, except the last, are reversible. (Modified from Potter, V. R.: *Medicine* 19: 441, 1940.)

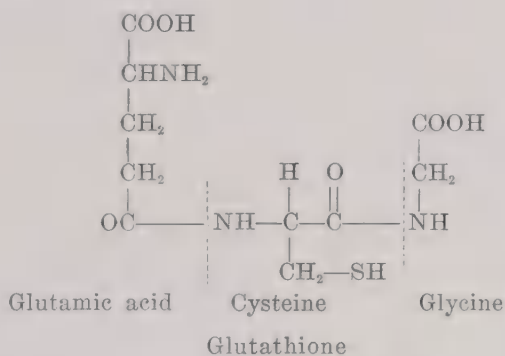
In this scheme only one cytochrome, cytochrome c, is shown, but it is possible that all three known cytochromes are linked together in an electron transport chain and operate in a system such as this. The sequence in the chain appears to be cytochrome b, c, a.

The various types of oxidizing systems outlined should not be considered inflexible categories. They are likely to be changed as our knowledge increases. Already it is known that some of the enzymes depend upon carriers which may be so closely linked to the enzymes that it is difficult to determine whether they are part of the enzyme molecule or not. It is evident that all the molecular oxygen that enters any biological oxidation appears as  $\text{H}_2\text{O}$ .

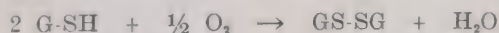
### Other Possible Agents

Mention should be made of certain compounds which possess the ability to be reversibly oxidized and reduced but which have not been shown to have any direct relation to the respiratory chains.

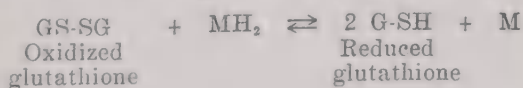
One very interesting reversibly oxidizable substance is glutathione. This substance, discovered by Hopkins, was found by him to be a tripeptide, glutamylcysteinylglycine.



Since the sulfhydryl grouping,  $-\text{SH}$ , is the effective part of the molecule, the formula is usually abbreviated to  $\text{G-SH}$ , the G, of course, representing all the rest of the molecule. Two of these molecules on oxidation are linked at the sulfur of each, yielding one huge molecule, just as two cysteine molecules may be joined to form a single molecule of cystine. The reaction may be indicated in this way



or in slightly alkaline solution it reacts with molecular oxygen. There is also the possibility that it may oxidize and reduce substances (metabolites or other carriers) according to the following reversible scheme:

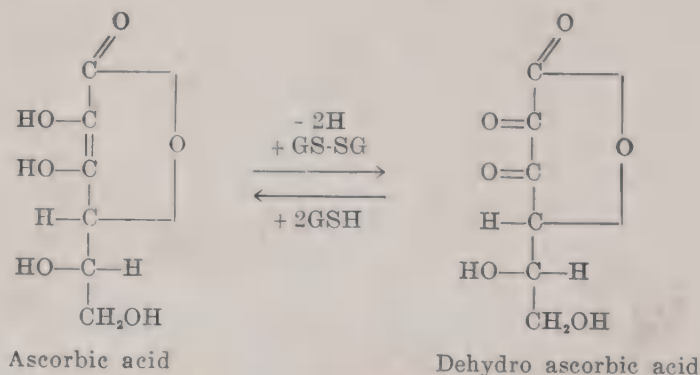


Glutathione is present in many body tissues and fluids. It was at first thought to be an extremely important oxidation-reduction agent. At the present time its role is quite uncertain. It apparently does not depend on any



enzyme for its activity but is dependent on traces of iron or copper. Although reduced glutathione is oxidized with great rapidity in tissues, it exists there for the most part in the reduced state. The reason for this is that it is reduced faster than it is oxidized. The reduction is not brought about by enzymes. Washed boiled muscle tissue will still reduce oxidized glutathione. The hypothesis has been advanced that this reduction is brought about by the  $-SH$  groups of the tissue proteins. Thus the tissues in general always have reduced glutathione present. This serves to maintain ascorbic acid in its reduced form in the tissues. In the same way it maintains those enzymes which possess  $-SH$  groups in their reduced form. These include many important ones involved in carbohydrate, nitrogen, and fat metabolism (Barron and Singer). No doubt other functions of glutathione will be discovered. It now is known to have other functions than oxidative ones. It is an activator of cathepsin and is also a coenzyme for glyoxalase. Thus it has an auxiliary oxidative function.

*Ascorbic acid*, vitamin C, is an extremely active reducing agent. This may easily be demonstrated in vitro and, in fact, this property is used in the quantitative determination of ascorbic acid in foods, in blood and tissues, and in excretions. It may act as a hydrogen carrier, giving its hydrogen to oxidized glutathione, for example, and in the oxidized state receiving hydrogen from reduced glutathione:



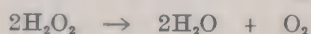
The exact role of ascorbic acid in oxidations in the body cannot be stated as yet. However, the fact that its presence in the diet is essential and that a high concentration exists in the adrenal cortex would indicate that it is of importance physiologically. It may be a factor in the conversion of heme to bile pigments, since it has been said to cause the transformation of hemoglobin to choleglobin, a biliverdin-protein complex. This is probably the first step in bile pigment formation. (Lemberg.)

It may also be involved in the metabolism of tyrosine. Artificial alkaptonuria in guinea pigs results when they are on a diet deficient in vitamin C but containing an excess of tyrosine. The subsequent administration of ascorbic acid reduces and even abolishes the output of homogentisic acid. Although ascorbic acid does not have the same effect in alkaptonuria in man, premature infants excrete p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids in the urine if vitamin C is not present in sufficient amounts in the diet. (Levine.)

The importance of thiamine has been stressed in Chapter 12 and some count of its actions in oxidative phenomena mentioned. As the pyrophosphate it appears to be a coenzyme for several enzymes, among which are a pyruvate-oxidase, a pure carboxylase, and a dehydrogenase-carboxylase. Whether or not thiamine always acts by the same mechanism is not known.

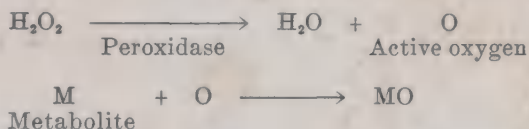
### Peroxidases and Catalases

In the end reaction of some of the chains, which have been described, the oxygen has been shown to unite with the hydrogen to form hydrogen peroxide. Peroxides thus might be expected to accumulate in large amounts in the tissues. Peroxides, being toxic, must be disposed of, and there are two enzymes capable of accomplishing this. The more important one is catalase. It is present in all animal cells but in varying concentrations. Its action is to decompose hydrogen peroxide, yielding gaseous oxygen:



Other peroxides are not attacked.

Peroxidase, in the presence of  $\text{H}_2\text{O}_2$ , catalyzes the oxidation of diverse phenols and aromatic amines. It has been pictured as an activation of the oxygen in such a way that the oxygen may directly oxidize the substrate.



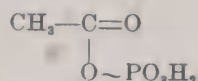
Many doubts whether enough hydrogen peroxide can occur in cells which contain catalase to bring about such oxidations and further whether there are sufficient phenols present for such reactions. There is also some doubt as to the occurrence of peroxidases in animal cells, because hemoglobin, cytochrome, and other substances react similarly and mask the presence of peroxidase. A peroxidase is found in milk, however.

### ENERGY PRODUCTION AND UTILIZATION

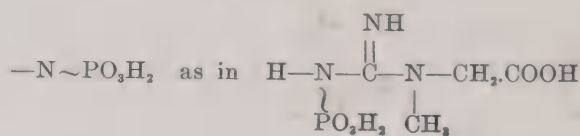
At the beginning of this chapter it was stated that one of the two main problems was how the energy derived from the oxidation of the substrate is utilized before being dissipated as heat. An explanation which has been advanced for this phenomenon will now be considered. It has long been known that the oxidation of triose and of pyruvic acid occurs only in the presence of organic phosphate. These three-carbon molecules may be considered typical metabolic units prepared by the body for oxidation. Phosphorylation and oxidation seem to be definitely linked together in physiological reactions. In the oxidation of the triose 3-phosphoglyceraldehyde, the first reaction supposed to occur is the addition of a second phosphoric acid to produce the hypothetical intermediary substance 1,3-diphosphoglyceraldehyde (Fig. 44). In the presence of an enzyme and a carrier, this is oxidized to 1,3-diphospho-

glyceric acid. This is the oxidation proper and although the reaction as a whole has liberated a small amount of energy, a large proportion of the energy of the oxidation is stored in the pyrophosphate bond created. This is a labile phosphate bond and is termed an "energy-rich" phosphate bond (Lipmann).

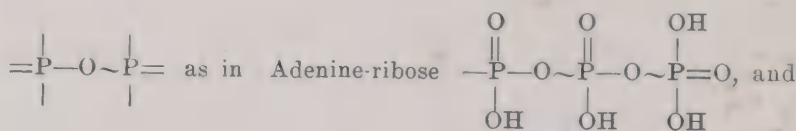
The energy-rich phosphate bond represents 9,000 to 12,000 calories per mole as contrasted with about 3,000 calories for the ester phosphate linkage. The 12,000 calories condensed in the energy-rich bond may be considered a *biological energy unit*. Removal of a phosphate linked to an alcoholic hydroxy group (ester phosphate) yields little energy. Through metabolic reactions phosphate may, however, become linked with carboxyl or certain other groups and form "energy-rich phosphate bonds." These bonds are shown with the symbol  $\sim$  to indicate this energy-rich type of linkage. Acetyl phosphate is



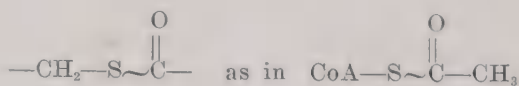
with one energy-rich phosphate bond. Other energy-rich bond types, with an example of a compound containing each, are as follows:



Creatine phosphate



Adenosine triphosphate



Acetyl Coenzyme A

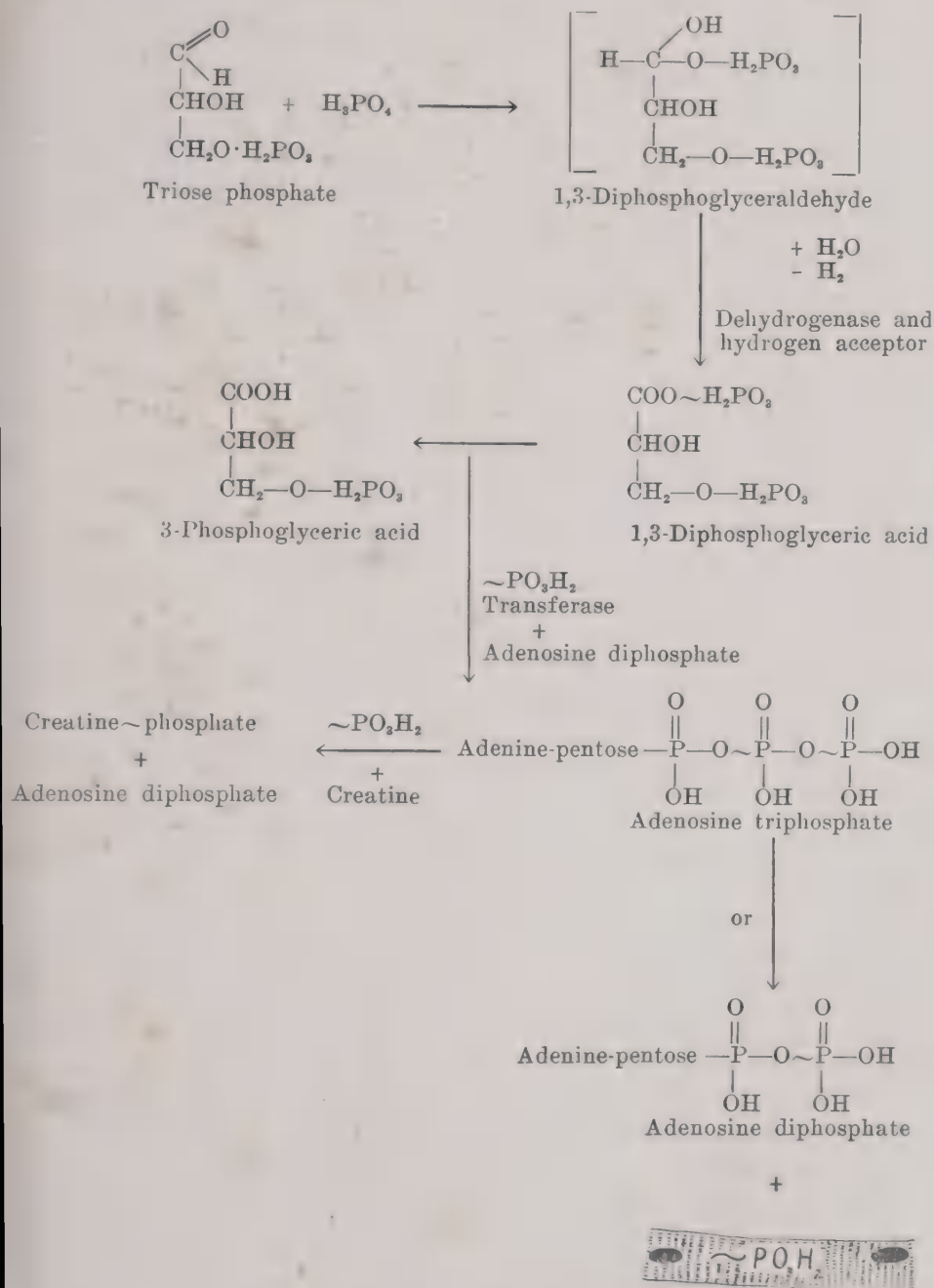
In the reactions shown in Fig. 44, the energy-rich phosphate bond has been inserted where it is postulated to be present. In the further catabolism of triose, the energy-rich phosphate bond is transferred from 1,3-diphosphoglyceric acid to adenylic acid or adenosine diphosphate by a specific enzyme, a transferase. At the same time this transfers about 12,000 calories to the adenosine molecule, which in turn can discharge this energy upon cellular structures for the performance of biological work, or it may transfer it to creatine. Most transphosphorylation reactions involve the adenylyl-pyrophosphate system which acts as a phosphate acceptor from substances like phosphopyruvate, acetyl phosphate, and 1,3-diphosphoglyceric acid. The adenosine polyphosphates can then act as phosphate donors to such organic substances as glucose, creatine, etc. The energy is finally made available when the energy-rich phosphate bond is broken, yielding inorganic phosphate, the dephosphorylated compound (adenylic acid, for example), and approximately 12,000 calories. The further



metabolic reactions in this series, with the formation of another energy-rich phosphate bond, are shown on page 424.

We may thus classify phosphate carriers into three groups.

1. Relatively inert phosphate carriers; e.g., ester phosphates, such as triose phosphate and hexose-phosphates. These carry on their phosphate bonds only 3,000 calories.



Energy-rich phosphate deposited on biological receptor (e.g., muscle fiber)

Fig. 44.—Energy transfers during catabolism of triose.

2. Active phosphate carriers, such as creatine-phosphate, 1,3-diphosphoglyceric acid, enol-phosphoric acid. These have about 12,000 calories condensed in their energy-rich phosphate bonds,  $\sim\text{PO}_4$ . They possess the property of transphosphorylation, which means the transfer of these energy-rich groups to other active carriers or to the third class.

3. Active phosphate carriers and dischargers. This comprises adenosine di- and triphosphates. They possess the properties of group 2, but in addition function in the performance of biological work. This includes muscle contraction, the maintenance of cell potential, etc. They also phosphorylate organic molecules such as hexose and triose, thus creating inert and active carriers.

Thus oxidative energy, from the oxidation of triose, is converted into phosphate bond energy and the adenylic system serves as the mediator of the transfers involved. When glucose is the phosphate acceptor, the system, once started, is self-perpetuating. The phosphorylation of glucose enables it to undergo oxidation by way of triose phosphate and pyruvate and this oxidation causes further phosphorylation of glucose. It should be emphasized that the energy is derived from oxidations, but the phosphorylations permit it to be concentrated in the energy-rich bond which is transferable through the agency of adenylic acid.

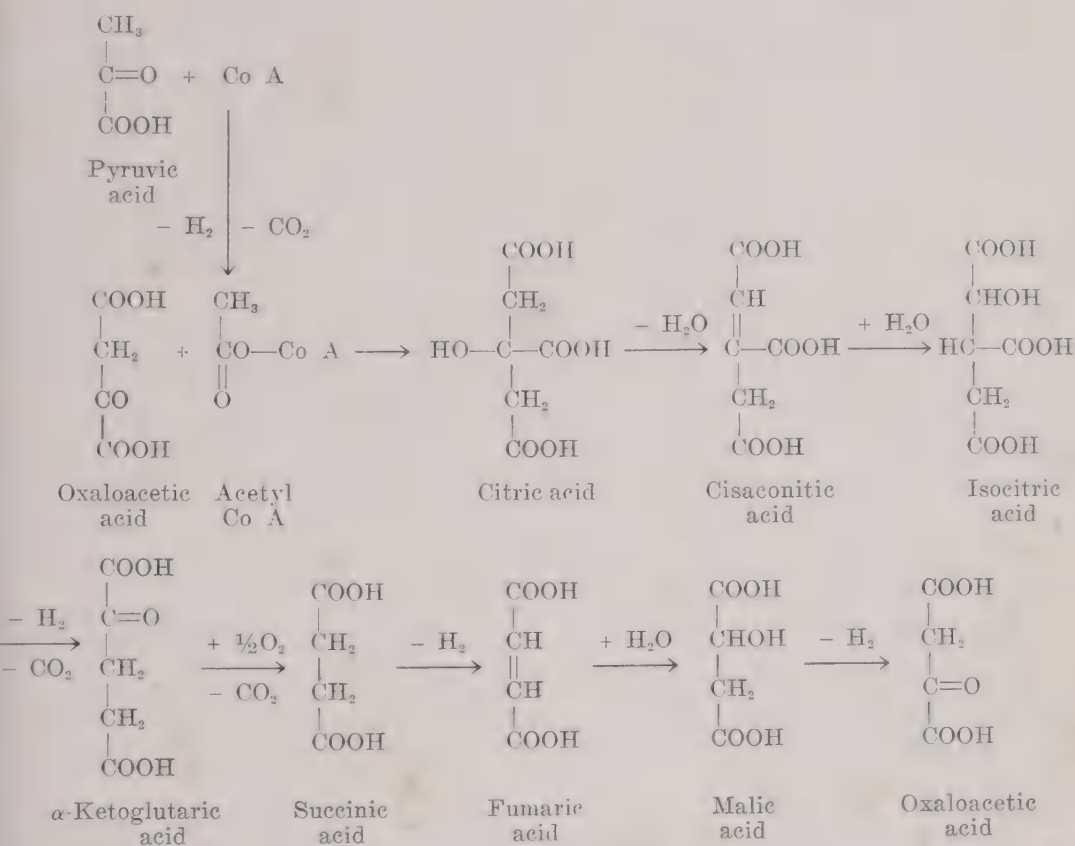
### Oxidative Decarboxylations

It is well known that the oxidation of metabolites in the body yields  $\text{CO}_2$ , water, and energy. In the preceding discussion it has been seen how oxidative reactions occur and that the oxygen which enters into reaction with the respiratory chain of enzymes appears as water. It has also been observed that high energy bonds may be formed in the course of the fermentative or anaerobic reactions. Up to this point, however, the degradation of the triose substrates to  $\text{CO}_2$  has not been explained, nor has the major production of energy-rich phosphate bonds, associated with the oxidation of foodstuffs, been described.

An appreciation of the importance of this phase of intermediary metabolism is largely due to the pioneer work of Szent-Györgi and Krebs. Szent-Györgi pointed out that there is no substance oxidized as rapidly by tissues as succinic acid. This acid has the unique property of possessing two carbon atoms which are both alpha and beta carbons. The same is true of the other C4 dicarboxylic acids. It is well known that such carbons are highly reactive and consequently would be expected to react rapidly under favorable conditions. A vast amount of experimentation led him to conclude that there exist two four-carbon dicarboxylic acid systems, each reversibly oxidizable. These when linked together, can transport hydrogen from the substrate metabolite to the cytochrome oxidase system.

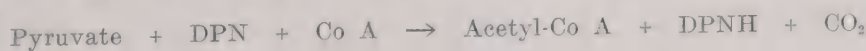
Krebs pointed out that pyruvic acid, arising from carbohydrate metabolism, and oxaloacetic acid undergo a condensation to citric acid in order to bring about oxidation of the citric acid and the regeneration of four-carbon

ids. The formulation of the tricarboxylic acid cycle by Krebs resulted from these and other investigations. It is given below, and in greater detail on page 423.



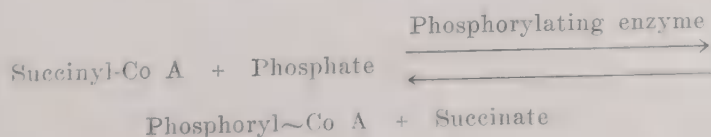
Oxaloacetic acid is thus an end product and can be used to condense with another molecule of pyruvic acid.

It should be noted that these reactions involve oxidative decarboxylations, which are complex reactions involving four essential agents: (1) diphospho- $\gamma$ -aminobutyrate and (2)  $\text{Mg}^{++}$  ions for the decarboxylation, thus creating an aldehyde intermediate, (3) DPN, for the oxidation of the aldehyde to an acyl compound, which is then accepted by (4) coenzyme A; namely:



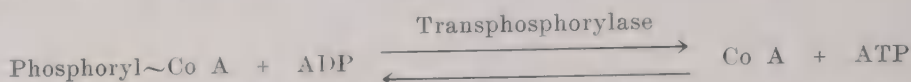
Similar reactions hold for  $\alpha$ -ketoglutarate decarboxylation wherein a succinyl-Co A compound is formed.

Coenzyme A consists of pantothenic acid, combined with a sulfhydryl compound, and ATP. Its formula is given on page 296. It is of great significance from the standpoint of energetic considerations, since it makes possible the coupling of phosphate with the oxidative decarboxylation reactions; for example:





The phosphoryl coenzyme A may then transfer its  $\sim \text{PO}_4$  to ADP.



From an energetic standpoint it is significant to note that a complete cycle of the Krebs reactions is associated with the creation of 16  $\sim \text{PO}_4$  groups. This is possible since the ratio of O:P may be 2 to 4 as contrasted with oxidative phosphorylation in glycolysis where the ratio is only 1. In the mitochondrial system the oxidation of each member of the cycle as well as the interaction of DPNH with flavin and of reduced flavin with cytochrome are all linked with phosphate esterification.

It is thus evident that by virtue of the fact that the enzymes concerned in the citric acid cycle are closely bound together in an oriented fashion within the particulate fraction of the cell, there is created a unity of structure and function. Green has introduced the term "cyclophorase system" to indicate the functional entity of this system of enzymes. The essentials of this concept are: (1) the unit of the system is an oriented or functional complex, and (2) this complex organization imparts catalytic potentialities above those of the individual components.

The catalytic activity of this system brings about the oxidation to  $\text{CO}_2$  and water of (a) members of the citric acid cycle, (b) fatty acids, fatty amines, and fatty aldehydes, (c) L-proline, L-alanine, L-glutamine and D-aspartate. The common feature in these reactions is the participation of the citric acid cycle of reactions.

**Metabolic Control.**—While in vitro chemical studies and energetic considerations indicate the possibilities and actual occurrence of certain reactions, it is evident that in the intact organism or cell a great deal of discrimination must be exercised in metabolism. Thus certain reactions must be favored or held in abeyance in accordance with the needs of the tissues. Any major error in such control would lead to functional, and eventually to anatomical, pathology. In short, homeostasis must depend upon and reflect a control of cellular enzymatic and respiratory activity. As examples of some of the various possible metabolic reactions which an amino acid may undergo, there are (1) conversion to peptides and proteins, (2) deamination to keto acids, (3) decarboxylation to amines, and (4) transformation to urea, or to some other nonprotein nitrogenous compound. In the case of pyruvate, we may have (1) reduction to lactate, (2) oxidation to acetate, (3) amination to form alanine, (4) carboxylation to oxaloacetate, (5) condensation with oxaloacetate to form citric acid, and (6) decarboxylation to acetaldehyde. The acetate conversion is of extreme importance, since it, in turn, may take part in (1) acetylation of amines and amino acids, (2) condensation to acetoacetate, (3) condensation to fatty acids, and (4) condensation to cholesterol, etc.

Among the limiting factors which determine the direction of metabolic reactions are those factors which influence the enzymes. They include: (1) the kind of enzymes present in the cells. This element appears to be controlled

a genetic basis. Experimental work indicates that removal or appearance of new enzymes may occur with mutations. (2) The concentration of enzyme. To a large extent this factor is a reflection of the type of substrate the cell is forced to metabolize. This ability provides a more subtle mechanism of adaptation to environmental changes than mutation and selective survival. (3) Location of the enzyme. Many of the respiratory enzymes are firmly bound to the different granular fractions of the cytoplasm. However, it is possible that physicochemical alterations in the protoplasmic colloid can lead to changes in the fixed spacial arrangement within the normal cell, and thus produce changes in the type and extent of metabolic reactions.

**Physiological Control.**—This aspect of metabolism is little understood. However, basically it must revolve around hormonal control of enzyme action. There is no evidence that hormones affect enzymes in homogeneous solutions. Cellular structure is apparently necessary for the manifestation of the effects of hormones. A suggestion of such control is found in the following examples:

(A) Hexokinase reaction. It appears that the phosphorylation of glucose is inhibited by a hormone of the anterior pituitary. This inhibition is negated by the action of insulin, the pancreatic hormone (Price). (See page 440.)

(B) Proline oxidation. Umbreit has shown that, in the adrenalectomized animal maintained on salt, the oxidation of proline by the kidney falls markedly, and this loss of proline oxidase is prevented or reversed by the administration of cortisone, a hormone of the adrenal cortex. Thus the cortisone appears to function in the formation of the enzyme, proline oxidase. No effect was observed on various other enzymes.

**Pasteur Reaction.**—Pasteur observed that fermentation varied inversely with the oxygen concentration. Under anaerobic conditions, yeasts fermented sugar, but upon the introduction of oxygen, fermentation ceased and oxidative reactions occurred. This phenomenon has been seen to occur in most forms of life and is called the Pasteur reaction. Although it has been extensively investigated, the basis of the reaction is still clouded in doubt. It is not even accepted that there is a specific Pasteur enzyme, which is postulated by some to be protected by thiol substances such as glutathione.

**Applications.**—A detailed listing of the possible applications of these studies is beyond the scope of this volume. However, it might be well to mention a few of the applications and potentialities that are known.

1. The insulin-anterior pituitary relationship described above.
2. It should be noted that many of the water-soluble vitamins appear to act as prosthetic groups of the oxidative enzymes.
3. There is strong indication that the sulfonamide drugs act by affecting the activity of respiratory enzymes in the bacterial cell. Gramicidin has been shown to dissociate phosphate uptake from glycolysis so that energetic reactions are impossible.
4. Substances have been discovered which appear to dissociate heat production from energy production. This provides an explanation of the mechanism of the action of various compounds, from "weight reducers" to bac-

terial toxins. Table XXXI lists a few of the agents affecting the coupling of oxido-reduction with energy utilization.

It thus appears that certain agents allow an exergonic step to occur without phosphorylation or that they catalyze the abnormal hydrolysis of phosphoric esters. This would account for the acceleration of oxidative and glycolytic processes and the failure of endergonic synthesis or work function.

TABLE XXXI  
AGENTS DISSOCIATING OXIDATION-REDUCTIONS FROM ENERGY UTILIZATION

AGENT	STIMULATES	INHIBITS
Dinitrophenol	Respiration and glucolysis	Maintenance of phosphocreatine
Dinitrophenol	Respiration and glucolysis	Assimilation
Dinitrophenol	Respiration and glucolysis	Sperm motility
Azide	Fermentation by yeast	Assimilation
Chloral hydrate	Respiration	Assimilation; luminescence
Gramicidine	Respiration	Assimilation; phosphate uptake

5. Examination of malignant growths from the standpoint of their energetic and enzymatic constitution reveals very unique features. It may be said that they exhibit very high values for both aerobic and anaerobic glycolysis and that they show deficiencies in their cytochrome—cytochrome oxidase systems. Based on these findings, chemotherapeutic methods of approach become possible.

6. Atabrine inhibits D-amino acid oxidase and also the oxidation of lactate, pyruvate, malate, or citrate. The site of action appears to be the inhibition of cytochrome reductase, glucose-6-phosphate dehydrogenase, and, to some extent, cytochrome oxidase. Addition of riboflavin overcomes the inhibition.

7. The formation of the acyl phosphate bond appears to be the initiating step in the oxidation of fatty acids. The level of adenosine triphosphate in tissue may control the relative amount of fat and carbohydrate oxidized.

In summary, the chemical and energetic metabolism of protein, carbohydrate, and fat meet at the active phosphate bond, which appears to be the final common path in biological energetics. While much remains to be explained, biochemistry is entering an era in which philosophical considerations of energy metabolism are opening new vistas in our understanding of the basic mechanics of the cell and of the organism.

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## Chapter 15

# NITROGEN METABOLISM

Metabolism comprises all the physical and chemical processes whereby protoplasm is produced and maintained. It includes "anabolism," the building up of substances to more complex structures, and "catabolism," the breaking down of substances by living things. It is a very broad term, since it includes both tissue and energy transformations. Under "nitrogen metabolism" only the various transformations and interconversions of proteins, purines, pyrimidines, creatine, and creatinine will be considered.

Nitrogen metabolism is necessarily quite different from carbohydrate and fat metabolism, because protein is the basis of protoplasm. Carbohydrates and fats do, to be sure, help to make up this living material but the needs of the organism for them are not so exacting. A particular protein of a given organ is probably unique in chemical constitution. It requires certain amino acids, and in definite proportions. Addis has shown that each organ or tissue has its own special reaction to different levels of protein in the diet. One organ acquires protein faster than another on a given percentage of dietary protein, indicating that each organ has its own optimum requirement.

## ABSORPTION

The proteins are digested in the alimentary tract almost completely to the amino acid stage. They are absorbed from the small intestine in this state, but there is a possibility that small amounts of peptides may also be absorbed. (Christensen, 1949.) Indeed it has been shown by the use of immunological methods that certain complete proteins are absorbed unchanged. However, usually the effect of digestion of proteins is to destroy their specificity, i.e., to break them down to amino acids, after which they are built up into entirely new proteins, characteristic of the animal which has ingested them.

If an animal is given a subcutaneous injection of a small amount of a particular protein, then, after a more or less definite interval, a second injection of the same protein is almost certain to produce grave symptoms and even death. The animal is sensitized by the first injection and the whole phenomenon is termed "anaphylaxis." Human beings sometimes experience the same thing; anaphylactic shock sometimes occurs after a second injection of some biological material if the timing is favorable for such a reaction. This may also explain why many individuals are hypersensitive to certain foods. That is, they may have been sensitized, perhaps by absorption from the intestinal canal of a minute amount of unchanged protein, and the toxic dose is similarly absorbed. It must be remembered that very small amounts are involved. Many persons experience symptoms of many kinds and degrees on eating certain foods. Sneezing, asthma, cutaneous rashes, headaches, vomiting, etc., are examples of the symptoms elicited by

specific foods such as strawberries, lobsters, onions—often when the food is present in such small quantities that the individual is unaware he has eaten any of it.

The principle of the method employed by Walzer to show that proteins may be absorbed unchanged is as follows:

By the intradermal injection of serum taken from certain hypersensitive patients, the skin of almost any individual can be passively and locally sensitized to a particular food, so that the subsequent ingestion of that food, under proper circumstances, will result in the formation of a wheal at the sensitized site. This local reaction is the result of the entrance into the blood stream of some of the ingested protein in an unaltered state.

By a more direct method Ratner and Gruehl have demonstrated the same phenomenon. The oral administration of a protein, not ordinarily in the diet, sensitized an animal to a subsequent feeding of the same protein. For example, cow's milk was fed to guinea pigs and after a suitable lapse of time, a second feeding was given. Anaphylactic shock resulted in a number of animals. However, it was not as violent nor as certain as when the second dose was given intravenously. This is not an accidental finding since it also occurred in a large proportion of human adults and children. The amount of protein so absorbed must be very minute and is of little moment from a nutritional standpoint. It is, however, of special significance in connection with the phenomenon of sensitization to specific proteins.

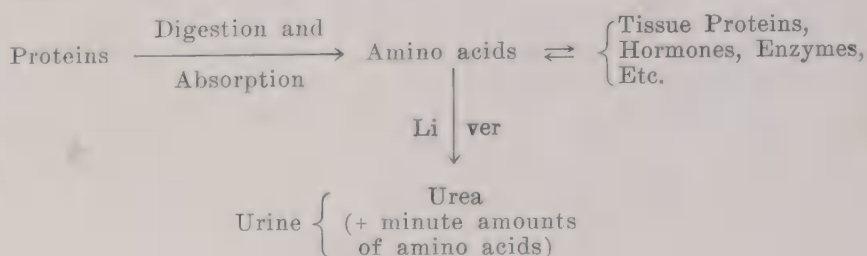
Although most of the amino acids occurring in nature belong to the L series, there are some that are D, particularly in microorganisms. All of the amino acids undergo metabolism, and certain, but not all, of the D variety may be used by the animal organism, in part at least. When this occurs, the D-amino acid is probably changed to the keto acid by oxidative deamination (see page 371), then reaminated to produce the L form, after which the normal processes occur. D-Tryptophan is one of those utilized to a large extent, while tyrosine is among those not physiologically available. (Oesterling and Rose; Albanese.)

## GENERAL PATH OF THE AMINO ACIDS IN THE BODY

The path of entry of the amino acids into the body is chiefly the portal circulation, where the amino acid levels are increased by the incoming amino acids. As the portal blood goes through the liver, the greater part of the extra amino acids, added by absorption, are removed by the cells of the liver. These amino acids enter these cells, despite the higher concentrations already there, apparently by an active transfer process. (Van Slyke and Meyer; Christensen, 1948.) Although the amino acid content of the liver rises faster and higher than that of other tissues after a meal, it falls off much more rapidly. This is largely because the liver is the only site of urea formation from amino acids, an activity which is accelerated by a high content of amino acids in the liver cells. Other portions of the amino acids are captured by other tissues. Here, too, they enter the cells against a higher concentration of amino acids, by some active transfer process. In the cells of the tissues in general, they replace corresponding amino acids of the protoplasmic protein and enter various other metabolic processes. Gradually the concentration of these compounds falls, as metabolic activities use them up, until the fasting levels are again reached. However, it should be understood that the various amino acids are constantly present in the blood stream, and it is clear that there must exist a homeostatic state for each amino acid, because, in the fast-



ing condition, the plasma level of each remains constant, just as much being returned to the plasma as is removed from it by cellular activity. The over-all relationships may be represented as follows:



### Nitrogen Balance

Probably the most interesting feature of the metabolism of the amino acids is the wide difference in their fate during growth on one hand and during adulthood on the other. In a normal adult, under usual conditions an amount of amino acids equal to the total amount taken into the body undergoes degradation and the nitrogen undergoes excretion each day. This means that the amount of nitrogen lost in a twenty-four hour period is approximately the same as that consumed, since the nitrogen ingested is chiefly protein nitrogen. In a growing child, in contrast, only a part of the amino acids suffers degradation; the remainder enters into the net synthesis of protein which is

TABLE XXXII  
EXAMPLE OF ADJUSTMENT TO CHANGES IN PROTEIN INTAKE\*

DAY	NITROGEN IN FOOD (GM.)	NITROGEN IN FECES (GM.)	NITROGEN ABSORBED (GM.)	NITROGEN IN URINE (GM.)	NITROGEN BALANCE (GM.)
<i>Experiment 1</i>					
Before	>16.96				
1	16.96	0.94	16.02	18.2	-2.18
2	16.96	0.94	16.02	17.0	-0.98
3	16.96	0.94	16.02	15.8	+0.22
4	16.96	0.94	16.02	16.0	+0.02
5	16.96	0.94	16.02	15.7	+0.32
<i>Experiment 2</i>					
1	14.40	0.70	13.70	13.60	+0.10
2	14.40	0.70	13.70	13.80	-0.10
3	14.40	0.70	13.70	13.60	+0.10
4	20.96	0.82	20.14	16.80	+3.34
5	20.96	0.82	20.14	18.20	+1.94
6	20.96	0.82	20.14	19.50	+0.64
7	20.96	0.82	20.14	20.00	+0.14

\*After von Noorden, from Sherman, H. C.: Chemistry of Food and Nutrition, ed. 6, New York, 1941, The Macmillan Co.

The subject was a young woman weighing 58 kilograms, at rest in bed. The first experiment is an example of adjustment to a lowered protein intake because it was known that the subject had previously been on a high protein diet. Equilibrium occurred after the second day. The second experiment shows the effect of increasing the protein intake. In this case three days elapsed before the subject was in nitrogen equilibrium.

characteristic of growth. The nitrogen which is lost from the body is the nitrogen of the urine, feces, perspiration, and those minor factors such as are included in the desquamation of the epidermis, the growth of hair and nails, salivary secretions, tears, etc. In striking a nitrogen balance, ordinarily only the nitrogen in the urine and feces is determined and is compared with the intake of food nitrogen. Usually the urinary nitrogen is estimated daily, while the feces, because of technical difficulties, are often collected over a three- or five-day period and the daily average estimated. A person is said to be in nitrogen equilibrium when the intake and output of nitrogen are equal to each other. A positive balance is that condition in which nitrogen is retained, and a negative balance is one in which more nitrogen is lost than is ingested. Positive balances occur during growth of children, convalescence of patients, and during pregnancy. In all these cases protein is being reconstructed and therefore the amino acid nitrogen is retained. For growth to occur in young animals, growth hormone and insulin are required. (See page 623.) These hormones also exert an influence in other types of positive nitrogen balance. Other hormones concerned in the regulation of protein metabolism are the thyroid hormone, testosterone, adrenal cortical hormones of the cortisone type, and ACTH. Negative balances are seen in starvation, malnutrition, fevers, after extensive burns or trauma, and postoperatively. Postoperative loss of nitrogen is a very common occurrence and is far greater than is usually appreciated. The severity depends on the extent of tissue damage and the degree of mobilization of amino acids for tissue repair. Under these conditions the body draws on its tissue and plasma proteins and hypoproteinemia may develop.

If an individual is in nitrogen equilibrium on a diet which is fairly constant and the amount of its protein and the level of protein intake is changed, he will, after a day or a few days, again become established in equilibrium at the new level. An interval of adjustment or "lag" is almost always seen. An example of this is given in Table XXXII.

### Uses of Amino Acids

In a general way it may be said that the amino acids are needed (1) for the synthesis of new protein in growth of tissues, formation of blood cells, and tissue fluids; (2) for replacing amino acids in tissue proteins; and (3) for building up those enzymes and hormones which are protein in nature or contain radicals possessed by some of the amino acids. Every cell contains many proteins, and since the cells of different tissues differ in their morphology, staining qualities, and, above all, functions, it is not strange that the number of individual proteins in one animal is very great. Multiply this by species differences, and we have an enormous number of diverse proteins. This is possible, of course, because protein molecules are huge and are made up of the twenty or more amino acids in varying proportions and arrangements. Abderhalden states that there could be 2,432,902,008,176,640,000 possible isomers of a polypeptide containing only one each of the twenty common amino acids, simply because of that many different arrangements. Chemically these would resemble each other remarkably. They would have the same percentage composition, give the

same reactions, and, of course, would have exactly the same hydrolytic products. They would differ in the arrangement of the "building stones" in the chain and would have distinct physiological and immunological properties. These polypeptides would have a molecular weight of about only 2,500. Since all the native proteins are much larger than that, it is evident that the number of different possible proteins is staggering. Consequently, the means Nature has adopted of breaking the food proteins down to their units, and reassembling them in the particular order and number required for each protein, seems to be a most advantageous process.

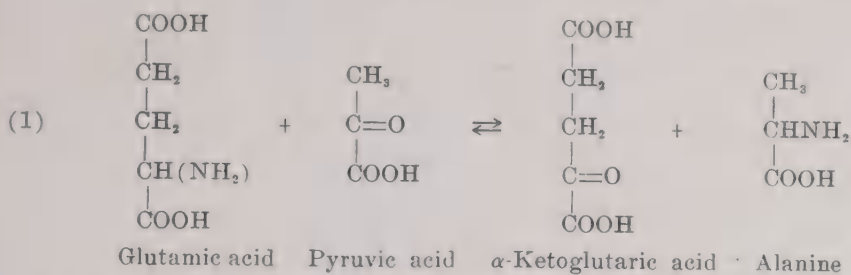
The organism does not store protein in the same way, or to the same extent, that it stores carbohydrate and fat. Nevertheless, during periods of high protein intake, considerable amounts of protein are stored in the liver and smaller quantities in the other tissues, probably built up into new tissue. That is, when the liver hypertrophies after high protein feeding, the increase is due to an addition of functioning liver tissue rather than to a deposition of inert storage material in the liver cells (Luck). This stored protein or new tissue protein is sometimes termed "reserve" or "labile" protein. This "reserve" protein is said to differ from the structural protein of cells in the readiness with which it may be utilized in times of protein shortage, such as starvation or hemorrhage. However, as stated previously (page 362), if animals are kept on different levels of dietary protein, the various organs will increase in weight and absorb protein at different rates, each organ responding optimally to a certain amount of protein in the diet. This indicates that the amount of new protoplasm in a tissue will depend on the type of tissue and on the supply of amino acids. The amount probably also depends on the kinds of protein eaten, that is, the assortment of amino acids available, but this has not been investigated. Moreover, new protoplasm can increase and decrease only within certain limits. Those organs, such as the liver, intestine, and kidney, which acquire protein rapidly, are the first to lose it when fasting occurs. In other words, "'labile' nitrogen is not distinguished primarily by a difference in composition but by its location" (Borsook and Dubnoff). Furthermore, it appears that nuclei of cells are spared longer than other parts of the cell. (Muntwyler.)

**Physiological Transformations of Amino Acids.**—In the laboratory, amino acids are relatively stable, but in the body they are highly reactive. They are built into the form of proteins with extreme rapidity. Certain ones take part in the formation of urea in the ornithine-citrulline cycle which will be discussed shortly. Some are easily changed to hormones such as adrenalin, insulin, and thyroxin. Recently the phenomena of *transamination*, *transmethylation* and *transpeptidation* have been described. They indicate how some of the amino acids take part in physiological phenomena.

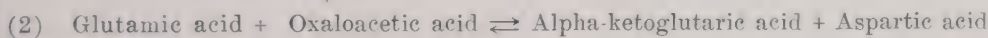
As the word indicates, transamination refers to the transfer of amino groups from one compound to another. L-Glutamic acid, under the influence of a specific enzyme, loses its  $\text{NH}_2$  to a keto acid, which thereby becomes an amino acid and the glutamic acid becomes a keto acid; namely,  $\alpha$ -ketoglutaric acid. The enzyme, transaminase, occurs in practically all animal tissues but in higher concentrations in heart muscle, brain, kidney, and testes. L-Aspartic acid may re-



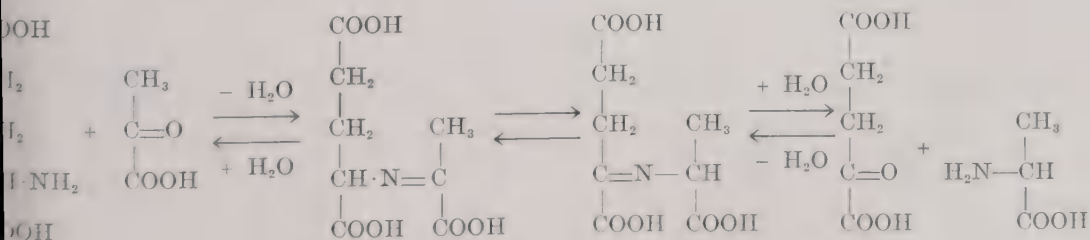
place glutamic acid, but the reaction is slower. The phenomenon was first discovered by Needham in 1927. One such reaction is as follows:



Twenty-two amino acids, besides alanine, are now known to be subject to transamination, but the glutamic- $\alpha$ -ketoglutaric acid system seems to be involved in each case, and a different enzyme is required for each. (Cammarata and Cohen.) A phosphate ester of pyridoxal, or, as the case may be, pyridoxamine, is the prosthetic group of transaminase acting as an amino group carrier (see page 295). In the reaction shown, pyruvic acid, a product of carbohydrate metabolism, is transformed into an amino acid, thus showing how the body is able to synthesize some of its amino acids. In this case it is one of the simplest ones. The reverse reaction indicates how the more complex glutamic acid might be produced from alanine and another intermediate in carbohydrate metabolism,  $\alpha$ -ketoglutaric acid. From another standpoint, transamination may be important. The nonnitrogenous compounds formed in these two-way reactions—pyruvic acid,  $\alpha$ -ketoglutaric acid, and oxaloacetic acid—are concerned in oxidation-reduction systems, some of which have been discussed. It thus appears that transamination may be a “shuttle” mechanism in which a few key protein and carbohydrate metabolites are interconverted as needed (Cohen). The most rapid transamination reaction is:



The reaction toward the right is twice as fast as the one in the opposite direction. The mechanism of transamination may involve the formation of intermediate complexes, by the condensation of the amino acid with the  $\alpha$ -keto acid. Rewriting reaction (1) we would have:



The methyl group of methionine,  $\text{CH}_3\text{-S-CH}_2\text{-CH}_2\text{-CH}(\text{NH}_2)\text{-COOH}$ , may likewise be transferred from that compound to suitable receptors. This phenomenon, transmethylation, was discovered and has been studied by duVigneaud and his colleagues. The result of transmethylation is, among other things, the formation of choline and creatine, both methylated compounds and both of utmost

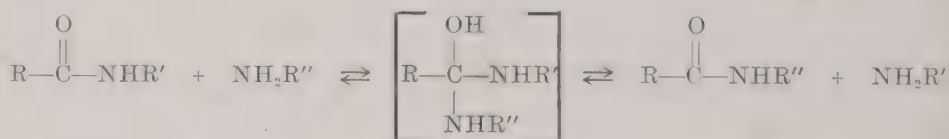
biological significance. Sulfur metabolism is also related to transmethylation, as will be seen.

Since the reaction

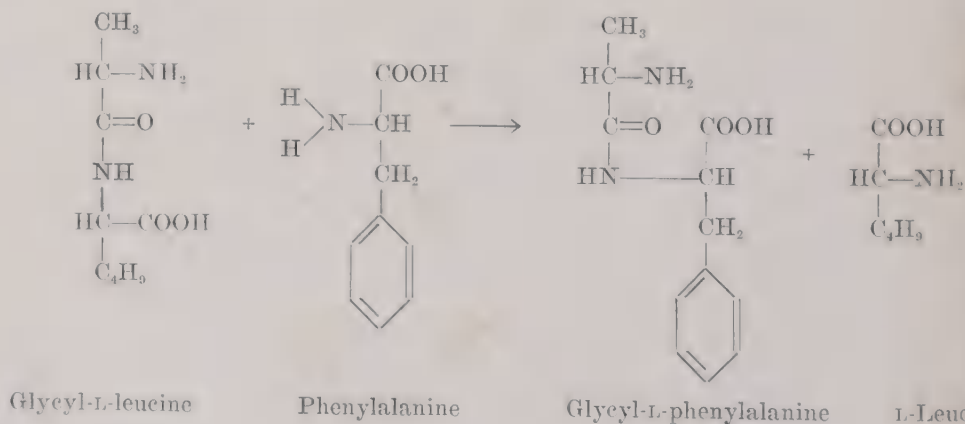


is a reversible reaction, theoretically the synthesis of peptides, and eventually proteins, is possible by means of the reverse reaction. However, the equilibrium lies far on the side of hydrolysis; i.e., toward the right. Synthesis is likely to occur, therefore, only if the peptide can be removed from the system rapidly enough, and, although this has been accomplished by using amino acids which yield insoluble peptides, it does not usually occur.

A different method of accomplishing syntheses of peptides, or of rearranging them, was discovered by Bergmann, Hanes, Fruton, Waelsch, and others; namely, *transpeptidation*. This is the term applied to the transfer of amino acids or peptides to amines, to other amino acids, or to peptides. Transpeptidations require little energy because the energy of the peptide bond which is broken is utilized for the synthesis of the new peptide bond. An intermediate compound is assumed to be formed, and in the new peptide the carboxyl group of the transferred amino acid is attached to the initial amino group. Carboxyl transfers, i.e., to the terminal carboxyl part of the receiving amino acid or peptide, are not yet known.

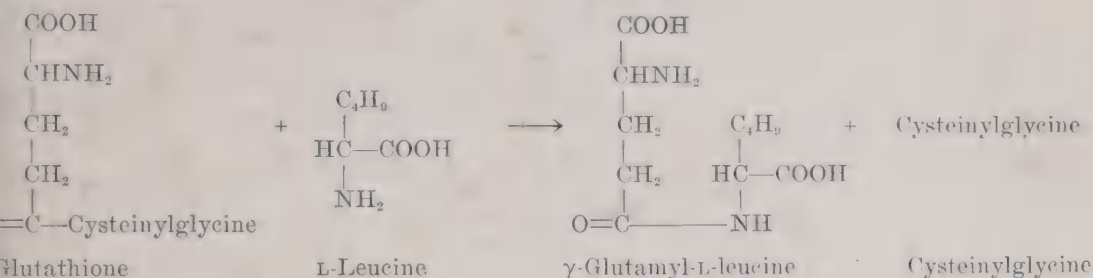


An example follows:

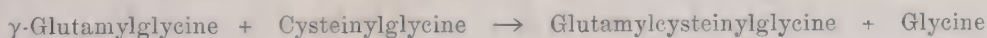


Although only short chains have thus far been shown to be formed, it is possible that long chain polypeptides are elaborated by such methods in nature. Among the enzymes that have been shown to take part in such transfers are cathepsin, trypsin, and chymotrypsin, and it is possible that all enzymes that catalyze hydrolytic reactions involving peptide bonds also catalyze transpeptidations.

Of particular importance is the  $\gamma$ -glutamyl radical, because of its incorporation in glutamine and glutathione. In mammalian kidney, liver, and brain there is a  $\gamma$ -glutamyl transpeptidase that transfers this group from glutathione to various amino acids; for example:



The reverse of this, namely, the synthesis of glutathione, has been accomplished in the presence of a suitable enzyme. (Fodor.)



If a new peptide bond is formed, a donor of high energy phosphate must be present. An example of this is the production of hippuric acid from benzoic acid and glycine. (See page 520.) ATP is required and is changed to ADP.

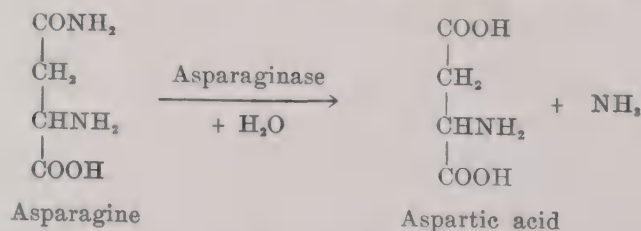
**Replacement of Amino Acids in Tissues.**—The amino acids free in the organism, both those in the cells and those in the extracellular fluid, are available to build new protein or rebuild the protein already present. The concept of endogenous and exogenous protein metabolism, supposedly based on firm foundations, must be modified and perhaps even abandoned. According to Folin, the tissues of the body basically have a more or less fixed amount of protein metabolism; that is, there is a "wear and tear" quota of tissue metabolism. This was called the "endogenous" metabolism and it was thought that it could be determined by ascertaining the minimum excretion of nitrogen on a diet containing no protein but sufficient carbohydrate and fat. When more than this minimum amount of protein was ingested, the excess over and above the endogenous nitrogen was ascribed to the breakdown of unused food, and was designated as "exogenous." The experimental basis for this concept was Folin's discovery that the urinary output of both creatinine and neutral sulfur was constant, regardless of the level of dietary protein. It appeared, therefore, that these constituents, but particularly the creatinine, resulted from cellular activity and that the lowest output of other nitrogenous products corresponding to them could likewise be considered to come from that basic cellular activity. The "worn-out" amino acids resulting from endogenous metabolism were carried to the liver and met the same fate as the exogenous, or excess, amino acids; i.e., they, too, were deaminized and the nitrogen excreted as urea.

In recent years a new theory of protein metabolism has been advanced by Borsook and Keighley. According to them, when a person is in nitrogen equilibrium, the breakdown of intracellular protein is continually going on but not at a minimum level. It bears no relation to the "wear and tear" of



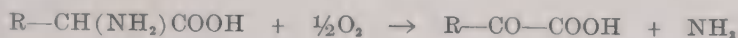
the cell but rather to the level of the amino acids present and available. It exceeds the Folin endogenous quota and is directly proportional to the level at which the nitrogen balance has been set by the intake of protein; that is, with a high protein intake, there will be a larger amount of interchange of amino acids than with a low intake. This interchange is a continuous process and the new theory is based on several types of evidence. Much of the evidence is indirect, but there is also some of a very direct character. Schoenheimer and his group synthesized amino acids with isotopic nitrogen,  $N^{15}$ , and have followed this nitrogen atom in its travels through the animal body. They have fed the amino acids containing heavy nitrogen to rats or mice and have then taken the tissues, hydrolyzed them, isolated the various amino acids, and determined their content of heavy nitrogen. This was accomplished by physical methods after the amino nitrogen was converted to gaseous nitrogen. The heavy nitrogen was found in the tissues very soon after feeding, and although present mostly in the form of the particular amino acid administered, it was also found in other amino acids to a considerable extent. The only amino acid in the tissues which did not exchange some of its nitrogen for "marked" nitrogen was lysine, although if lysine containing heavy nitrogen was fed, it could yield its nitrogen for the formation of other amino acids. That is, as far as lysine is concerned, the process is in one direction only. The transfer of  $NH_2$  occurred by oxidative deamination, transamination, and also by other procedures, the natures of which are unknown. It seems that every protein molecule in the body is frequently renewed, and this does not imply a wearing out of the protein molecule or any part of it but rather a dynamic equilibrium between the amino acids in the tissues, in plasma proteins, and those circulating in the blood and body fluids. To accomplish this, peptide linkages are being opened and closed continually at a very high speed with the removal and replacement of amino acid molecules, or protein molecules are being hydrolyzed and resynthesized.

**Deamination.**—It has been stated that after absorption from the alimentary tract, a large share of the amino acids is picked up by the liver and is soon deaminated. Probably amino acids exchanged in other tissues for the fresh ones circulating in the blood make their way back to the liver, because this organ is the chief site of deamination in the body. The kidney and other organs share this function but only to a minor degree. There are two general types of reaction involved, each catalyzed by a specific enzyme. The first is a simple hydrolysis of an amino acid amide, such as glutamine or asparagine, yielding the corresponding amino acid, glutamic or aspartic, respectively.



These two amino acid amides are present in the proteins as such to some extent. They also are formed in certain tissues by the addition of ammonia to the amino acid under the influence of an enzyme, glutaminase.

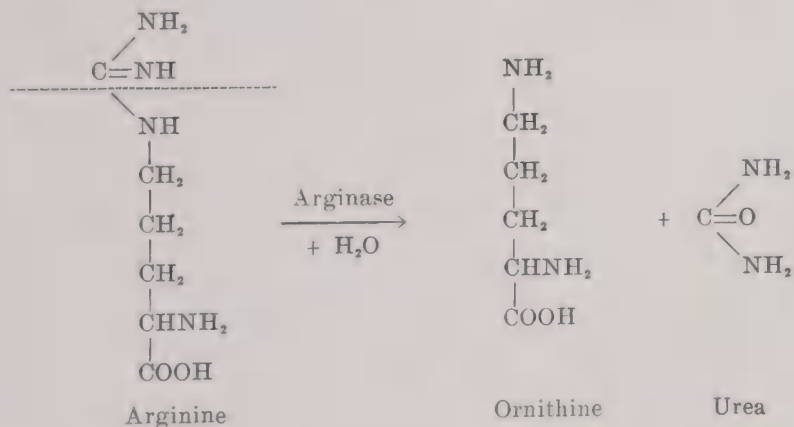
The second type of reaction is an oxidative deamination, which converts  $\alpha$ -amino acids into their corresponding keto acids and releases ammonia:



The enzyme is L-amino acid oxidase and acts in the presence of atmospheric oxygen which has been carried to the tissues by hemoglobin. This second type is considered the general reaction by which most amino acids are deaminated. The following L-amino acids have been shown to be oxidized by this enzyme: leucine, phenylalanine, norleucine, isoleucine, valine, cystine, histidine, tyrosine, methionine, alanine, and tryptophan. (Blanchard.) The nonnitrogenous residue will be considered later. Now we are concerned with the fate of the ammonia produced by both of these reactions.

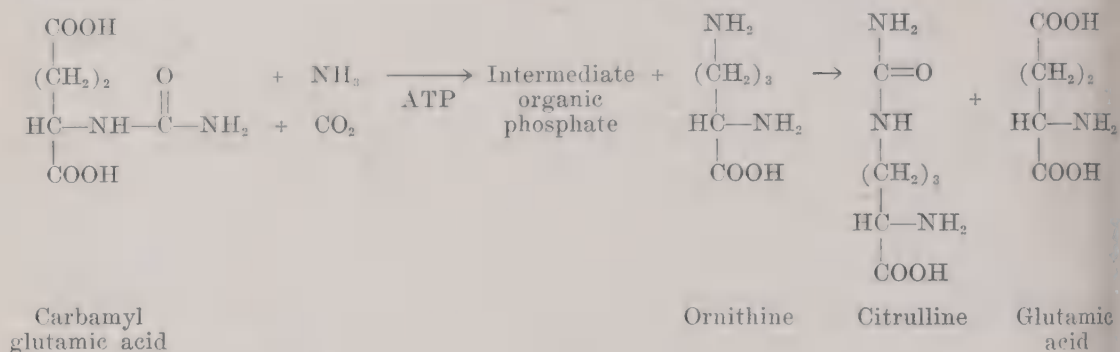
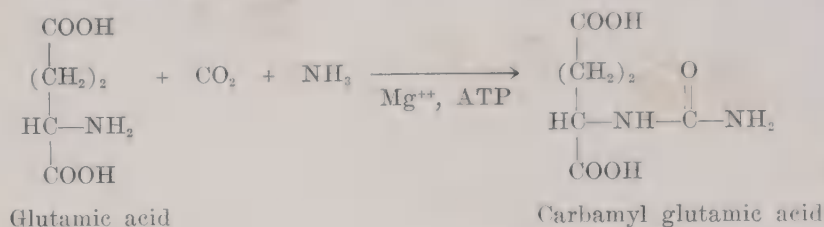
### Urea Formation

The ammonia formed by deamination is converted into urea in the liver. This had been suspected for a long time, but Bollman, Mann, and Magath confirmed it in 1924 by showing that hepatectomized dogs were unable to form urea. After such an operation the amino acids and ammonia accumulated in the blood, but the urea of the blood and tissues decreased in concentration. If the kidneys were ligated in such animals, the blood urea remained at a constant level. It is therefore apparent that the liver, and only the liver, produces urea. Furthermore, we know that there is present in the liver an enzyme, arginase, which splits urea off of arginine, leaving ornithine, another amino acid, as a residue.

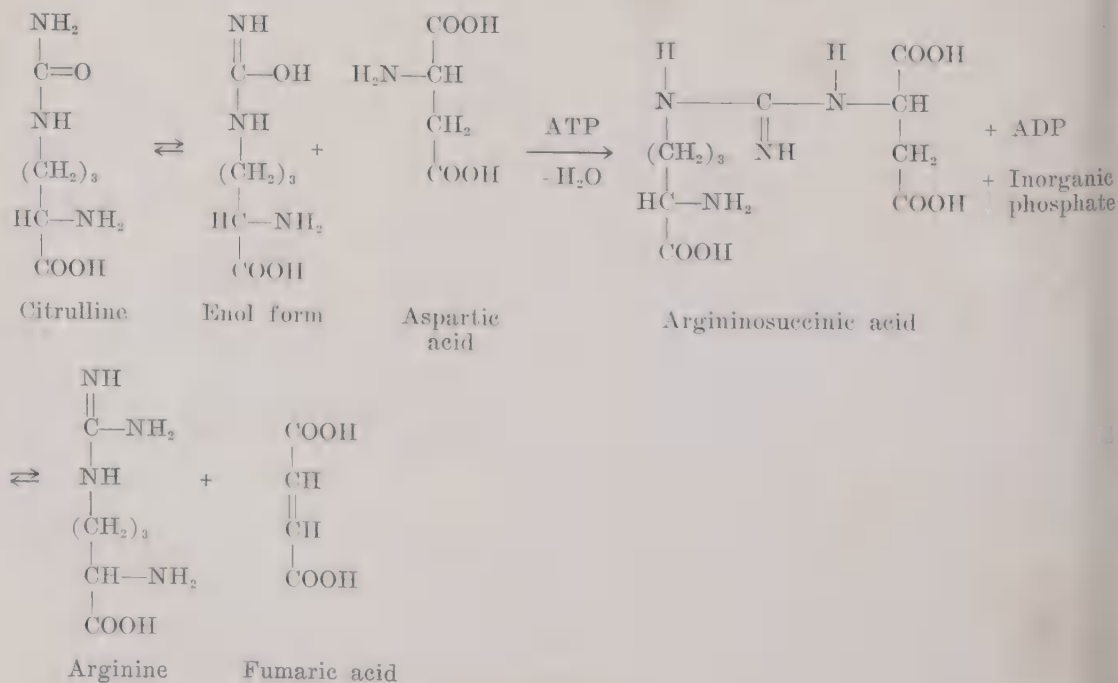


Krebs and Henseleit studied this reaction by the use of slices of liver tissue in an oxygenated nutrient medium containing ammonium salts. They were able to obtain urea formation only with liver tissue, thus confirming the fact that liver is the sole site of urea manufacture. The amino acids, ornithine and citrulline, catalyzed the reaction to a marked degree. Later it was observed that glutamine or glutamate also had a stimulating effect on urea formation. It was soon discovered that the formation of urea from arginine, as shown above, is really the last of a series of enzymic reactions. These include the synthesis of citrulline from ornithine and glutamate, the synthesis of arginine from citrulline, and the splitting off of urea from arginine.

(1) It was found by Cohen and Grisolia that glutamate accepts  $\text{CO}_2$  and  $\text{NH}_3$  and thus forms carbamyl glutamic acid. This requires ATP. One would have supposed that this carbamyl group would next be transferred to ornithine to yield citrulline; instead, a more complex intermediate is formed, which transfers a different  $\text{CO}_2$  and a different ammonia to citrulline.



(2) Citrulline, after enolization, reacts with aspartic acid to form an intermediate addition compound (now known to be argininosuccinic acid), which yields arginine and fumaric acid. ATP is required in the first of these two steps, possibly to phosphorylate the hydroxyl group of the enol form of citrulline. (Ratner and Petrack.)





(3) The arginine formed is decomposed by arginase with the formation of urea and ornithine. The ornithine is again ready to take up  $\text{CO}_2$  and  $\text{NH}_3$  to form carbamyl glutamic acid. In this cycle it is seen that two molecules of ammonia and one of carbon dioxide are taken up. The ammonia, it should be remembered, is derived from amino acids, and the  $\text{CO}_2$  from the metabolism of carbohydrates, fats, or the nonnitrogenous residue of proteins.

Urea formation involves at least seven enzyme reactions—three in the formation of citrulline, three in the formation of arginine, and one in the formation of ornithine with the concomitant splitting off of urea. Two of these seven enzyme reactions are endergonic, and about 13,800 calories of free energy are released in the formation of urea from  $\text{CO}_2$  and  $\text{NH}_4^+$ .

A scheme of this cycle is shown in Fig. 45.

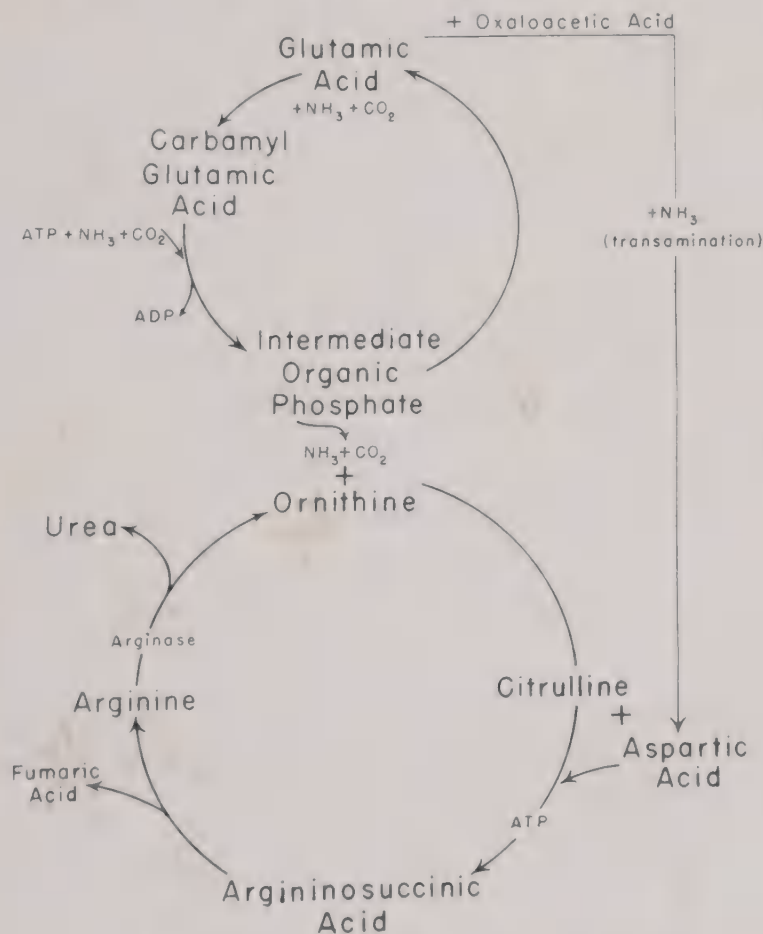


Fig. 45.—Scheme showing formation of urea.

**Amount of Urea Excreted.**—Urea is the chief nitrogenous end product of amino acid metabolism. Small amounts are also derived from the breakdown of other products, but these are negligible. On a normal or a high protein diet the urea (about 25 to 30 Gm.) comprises the greatest part of the urinary nitrogen; from 85 to 92 per cent of the total nitrogen is urea nitrogen. On a low protein diet the urea nitrogen forms a smaller fraction. It

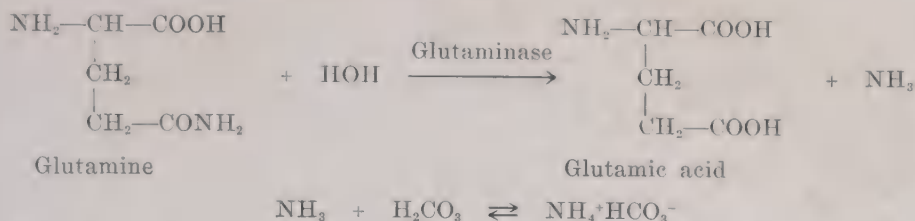
may be as low as 60 per cent of the total nitrogen. The reason for this is that certain nitrogenous constituents of the urine, such as uric acid, ammonia, and creatinine, are present in comparatively small amounts even on a high nitrogen diet, and when the protein intake is lowered, they continue to be excreted in almost the same amount. They arise from other phases of metabolism not directly concerned with the breakdown of protein.

Urea itself has no very marked physiological effects. It has some diuretic action. Consequently on a high protein diet the volume of urine eliminated tends to be increased. This may be the reason for the feeling of thirst one often experiences after a very hearty meal.

### Ammonia Formation

Ammonia formation from a quantitative standpoint is quite unimpressive when compared with urea. It amounts to about only 2.5 to 4.5 per cent of the total nitrogen under ordinary conditions. However, it is of considerable importance as a neutralizing agent for acids. As such it conserves fixed bases, such as Na, K, and Ca. If an increased amount of acid is ingested, more ammonia is formed to neutralize it and thus the loss of these elements is prevented. The same is true when an acidosis occurs and the reverse when there is an alkalosis (see Chapter 20).

Nash and Benedict showed that ammonia formation occurs in the kidney. They ligated both kidneys and demonstrated that the ammonium salts of the blood did not accumulate as they would have done if  $\text{NH}_3$  were formed in some other organ. They also showed that the blood of the renal vein contained more ammonium salts than blood from other parts of the circulatory system. That indicated that the kidney manufactures ammonia, a small amount of which diffuses into the blood for neutralizing purposes. Most of it is eliminated by the kidney and forms the ammonia fraction of the urine. The source of ammonia has been the subject of much investigation as well as controversy. The use of isotopic compounds has helped to clarify the matter. The feeding of isotopic ammonia was followed by the excretion of isotopic urea, almost exclusively. Similarly the administration of isotopic urea was followed by the excretion of isotopic urea. Ammonium salts, which might arise in digestion to a slight extent, and urea are therefore both excluded as sources of urinary ammonia. However, when various amino acids containing heavy nitrogen were fed, a large proportion of it was found in the urinary ammonia. Probably the urinary ammonia is formed by direct deamination of the amino acids without urea as an intermediate. Van Slyke and his colleagues have proposed the amino acid amide, glutamine, as the chief source of ammonia. Dogs were prepared with kidneys explanted; that is, transferred to positions under the skin. In this way blood could be withdrawn from the renal vein by skin puncture. By special methods of analysis, they found that glutamine was the major source of ammonia with alpha-amino acids a minor one. Glutamine makes up about one-fourth to one-fifth of the free amino acids present in plasma.



**Fate of the Nonnitrogenous Residues.**—The portion of the amino acid remaining after deamination is transformed into glucose, into acetoacetic acid, and other related compounds, and into other products. The structure of the amino acid will determine into which substance it will be changed. The evidence for the data on this point has been obtained chiefly from experiments on dogs or other animals which were made diabetic either by the removal of the pancreas or by the injection of phlorizin. Such diabetic animals excrete glucose even when starved or on a diet containing only protein. It is evident that when all the fat and glycogen stores have been used up, any glucose excreted in the urine must have been derived from protein. Since the nitrogen content of proteins is known (approximately 16 per cent), a determination of both urinary nitrogen and glucose should indicate the proportion of sugar which can be derived from protein. This has led to the formulation of the G:N ratio, formerly called D:N or dextrose:nitrogen. Minkowski's figure for the G:N ratio in depancreatized dogs was 2.8. The G:N ratio in phlorizin diabetes was raised at 3.65 by Graham Lusk (see page 432), and it has been assumed that the latter value is the better one to use. Although a great deal of doubt has been cast upon the accuracy of this figure, and, indeed, upon part of the theoretical basis for it (Soskin and Levine), its use may be continued until the subject is in a less confusing state. Moreover, from a practical standpoint the results obtained are very useful. Now, if the G:N ratio is 3.65 on a protein diet, the indication is that the amount of glucose derived from 100 Gm. of protein ingested is 58 Gm.

$$\frac{\text{G (Glucose of the protein)}}{\text{N (Nitrogen of the protein)}}, \text{ or } \frac{x}{16} = 3.65. \quad x = 3.65 \cdot 16 = 58.4$$

The process of forming glucose from protein is called "gluconeogenesis" and, hence, the gluconeogenic value of proteins in general is 58 per cent. When diets are planned for diabetic patients, and the approximate amount of potential glucose in the food must be known with some degree of accuracy, this figure is of value. Fifty-eight per cent of the protein ingested is added to the amount of carbohydrate for a "total glucose" value of the diet. (About 10 per cent of the fat is also included; this represents the glycerol fraction, which is easily converted into carbohydrate.) This figure, 58 per cent, it must be understood, represents the glucose derivable from a mixture of proteins. Single purified proteins yield different amounts of glucose and nitrogen. Janney gives the following results: Casein, 48 per cent; ovalbumin, 50 per cent; serum albumin, 55 per cent; gelatin, 68 per cent; fibrin, 53 per cent; casein, 65 per cent; gliadin, 80 per cent; and zein, 53 per cent. These divergent figures are due to the differences in composition of the proteins. Feeding pure



amino acids to diabetic animals results in the interesting observation that only certain ones give rise to glucose in this way. It has been shown that:

1. The amino acids which yield glucose are all those which contain two-, three-, four-, or five-carbon atoms.
2. Of those with more than five carbons, arginine and histidine are the only ones which give sugar.
3. All the straight-chain amino acids except lysine furnish sugar.
4. Proline and histidine are the only amino acids with cyclic formulas which are known to be convertible to glucose.

Lysine is in a special class in this as well as other ways and presumably has a special type of metabolism. Regarding arginine, it has just been seen how arginase cuts it down to a five-carbon chain in the liver. It is therefore not surprising that it follows the same route as the other smaller straight chains and is converted into glucose.

Thus the following amino acids give rise to sugar: glycine, alanine, valine, serine, threonine, cysteine (and cystine), methionine, aspartic acid, glutamic acid, arginine, histidine, and proline.

The mechanism of this conversion to glucose is not known. However, it has been seen that keto acids are the first step. The simplest keto acid, pyruvic acid, is a well-known intermediate in all carbohydrate transformations, but it is not known whether the longer keto acids must be cut down to pyruvic first or not. It is possible that this is so because only three of the carbon atoms in each glucogenetic amino acid go to form sugar. In the case of glycine, of course, only two are so converted. There is some evidence that the glucogenetic amino acids may not in all cases be actually converted into glucose. The glucose formed may come from some other metabolite, which the amino acid spares. Gurin and Wilson found that tagged alanine, when administered to phlorhizinized dogs, resulted in a formation of glucose, to be sure, but the glucose was not tagged. Similar results have been obtained by other workers.

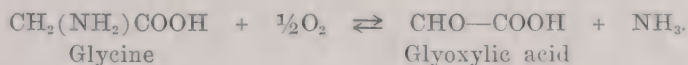
Leucine, isoleucine, hydroxyproline, phenylalanine, and tyrosine give rise to acetoacetic acid, and probably some of the others also do. The mechanism of this process is another which is not understood as yet. It may also be mentioned in this connection that since many amino acids yield glucose, and carbohydrates are convertible to fats (Chapter 17), the proteins indirectly may be fat-formers. The opposite to glucose formation from proteins also occurs; i.e., glucose and many related compounds can be converted into the carbon chains of amino acids. For example, when sucrose, embodying  $C^{14}$  was fed to mice, the tagged carbon was found in several of the nonessential amino acids of the tissues. (Steele.)

### Metabolism of Some Individual Amino Acids

Most of the discussion of amino acid metabolism up to this point has been concerned with these compounds as a class. It is now necessary to consider them individually, or in groups, and they will be taken up in about the same order as they were listed in Chapter 5. The problem to be studied is how the amino acids are handled by the liver or other tissues, when they are not being

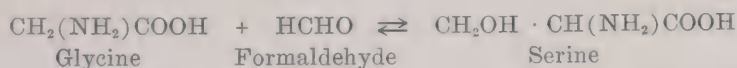
ult into protoplasm, and, further, to see whether any of these compounds are of special interest physiologically or clinically.

**Glycine.**—If the general reaction for the oxidative deamination of amino acids is applied to glycine, we have



This reaction has been demonstrated to be catalyzed by glycine oxidase, an enzyme present in liver and kidney tissue. (Ratner.) The enzyme is a flavoprotein. Glyoxylic acid may be decarboxylated to yield formaldehyde and carbon dioxide, both of which take part in many biochemical reactions.

An interesting possible pathway involves the participation of glycine in the synthesis of serine, which could then be converted to pyruvic acid (page 78), a carbohydrate metabolite (Sakami). Serine may also be converted to glycine.

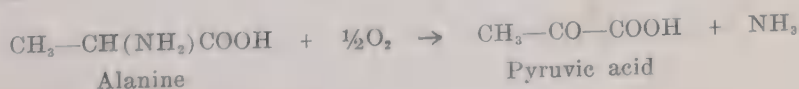


Glycine is one of the substances necessary for the formation of creatine, and creatine is an essential in muscle physiology. Consequently when it was found that the creatine-creatinine metabolism was disturbed in certain conditions involving the musculature, the feeding of glycine was tested. The results are conflicting. In one of these conditions, "myasthenia gravis," in which disturbances in creatine metabolism appear to be of secondary importance, the feeding of glycine sometimes has favorable results, but not in all cases. In "progressive muscular dystrophy," on the other hand, which invariably is accompanied by abnormal excretion of creatine, the use of glycine therapeutically has been generally disappointing.

Glycine is also a part of the bile acid, glycocholic acid, and the tripeptide, glutathione, which has certain oxidation-reduction functions. It has been shown to be transformed into ribose, fatty acids, aspartic acid, purines, pyrimidines, and into the porphyrin structure of heme. These changes are probably attributable to the fact that glycine is a small molecule, like formic and acetic acids, into which glycine may also be converted, and which are known to enter into the biosynthesis of many complex compounds.

When benzoic acid, or its salts, are included in the diet, glycine conjugates with it to form hippuric acid. This is a "detoxicating action." It occurs even when no glycine is present in the diet; hence glycine must be readily obtainable from other amino acids and is therefore one of the dispensable amino acids. Although a dispensable amino acid from the standpoint of its nonrequirement in the diet, the above facts indicate that it is definitely needed for many biological activities.

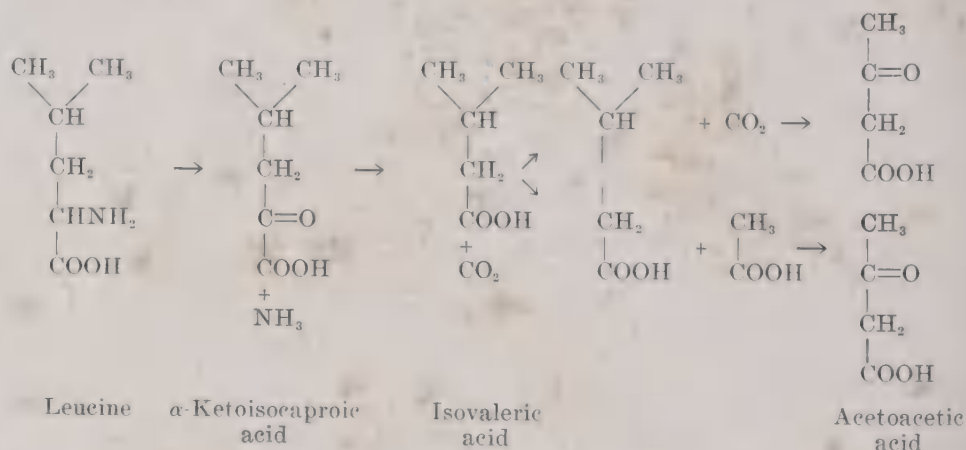
**Alanine.**—Alanine, by the general oxidative deamination reaction, forms pyruvic acid.



Under the influence of L-amino oxidase this reaction proceeds very slowly, and it is doubtful if this is the major metabolic pathway for alanine. It was shown, however (page 367), that, by transamination, alanine can be converted into pyruvic acid, the same end-product as in oxidative deamination. Pyruvic acid is an intermediate in the metabolism of carbohydrates. Here is a very clear relationship between the proteins and carbohydrates. As has been seen, alanine, when fed to diabetic animals, is converted into glucose. In normal animals it is similarly converted, but is then utilized.

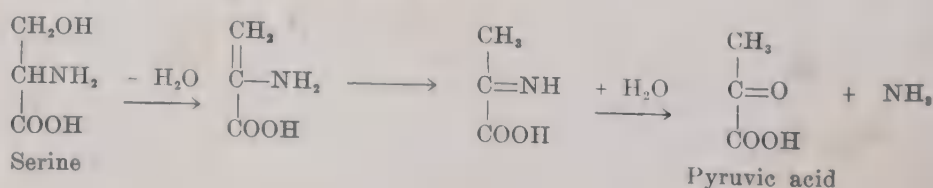
**Valine.**—It has been shown that valine follows the common path of deamination, yielding the corresponding keto acid. Transamination would have the same result. Besides the fact that three of its five carbons are converted to glucose in the phloridzinized dog (Rose), little is known of its fate in catabolism.

**Leucine and Its Isomers.**—Leucine, isoleucine, and norleucine all undergo oxidative deamination or transamination to form the keto acid. (Norleucine is the straight chain isomer.) From the investigations of Coon and Gurin, it appears that the further degradation of leucine follows a break in the carbon chain between the  $\beta$  and  $\gamma$  carbons. Both fragments yield acetoacetate. This was determined by administering leucine containing  $C^{14}$  in various positions in the molecule, or incubating it with liver slices, and discovering the exact location of the tagged carbon of the acetoacetate formed in each case.



The fate of the acetoacetate acid will be discussed under fat metabolism, where it is a major product. This is another example of the interrelationship of protein, fat, and carbohydrate metabolism. Little is known of the pathways of degradation of isoleucine and norleucine beyond the keto acid stage.

**Serine.**—The anaerobic deamination of serine has been demonstrated by Chargaff and Sprinson. In this case the reaction differs from the general one heretofore considered:





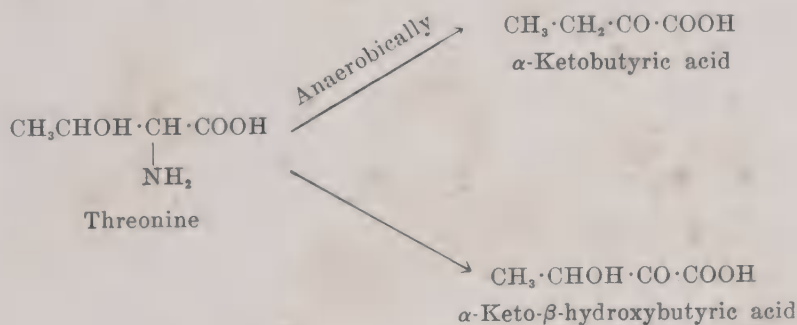
These investigators found the above reaction to occur in liver extracts. The possibility of the production of  $\beta$ -hydroxypyruvic acid by an oxidative deamination is not excluded. Both pyruvic acid and  $\beta$ -hydroxypyruvic acid can easily enter into metabolism, and pyruvic acid would likewise be formed in the transamination of serine. Isotope experiments indicate that the carbon chain of serine can be converted to cystine (Stetten) (see page 386). Cystine, di-cysteine, and cysteine has the same number of carbon atoms as serine.

It will be remembered that serine is also a constituent of one of the phosphatides found in brain. Furthermore it can give rise to ethanolamine, which is one of the constituents of another phosphatide. Thus we see links between protein and lipid metabolism.

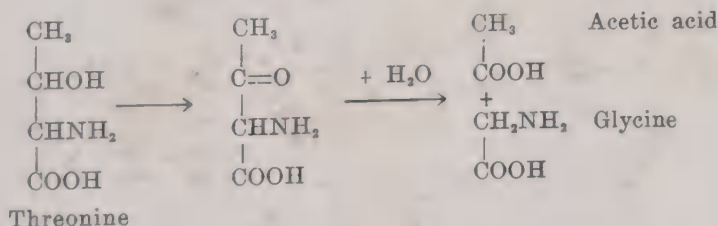


Serine also takes part in the biological synthesis of tryptophan in the mold *Aspergillus niger*.

**Threonine.**—Threonine is expected to be handled similarly to serine, since it also is a hydroxy amino acid.



By an indirect method, another path for threonine has been indicated (Knoop). It is an oxidation at the beta carbon, preliminary to a splitting of the four-carbon chain in half.



**Phenylalanine and Tyrosine.**—The animal body cannot synthesize the benzene ring, and these two aromatic amino acids are the chief source of it in the body. Phenylalanine is converted to tyrosine in the body, and this appears to be the first step in its metabolism; the reverse reaction is not

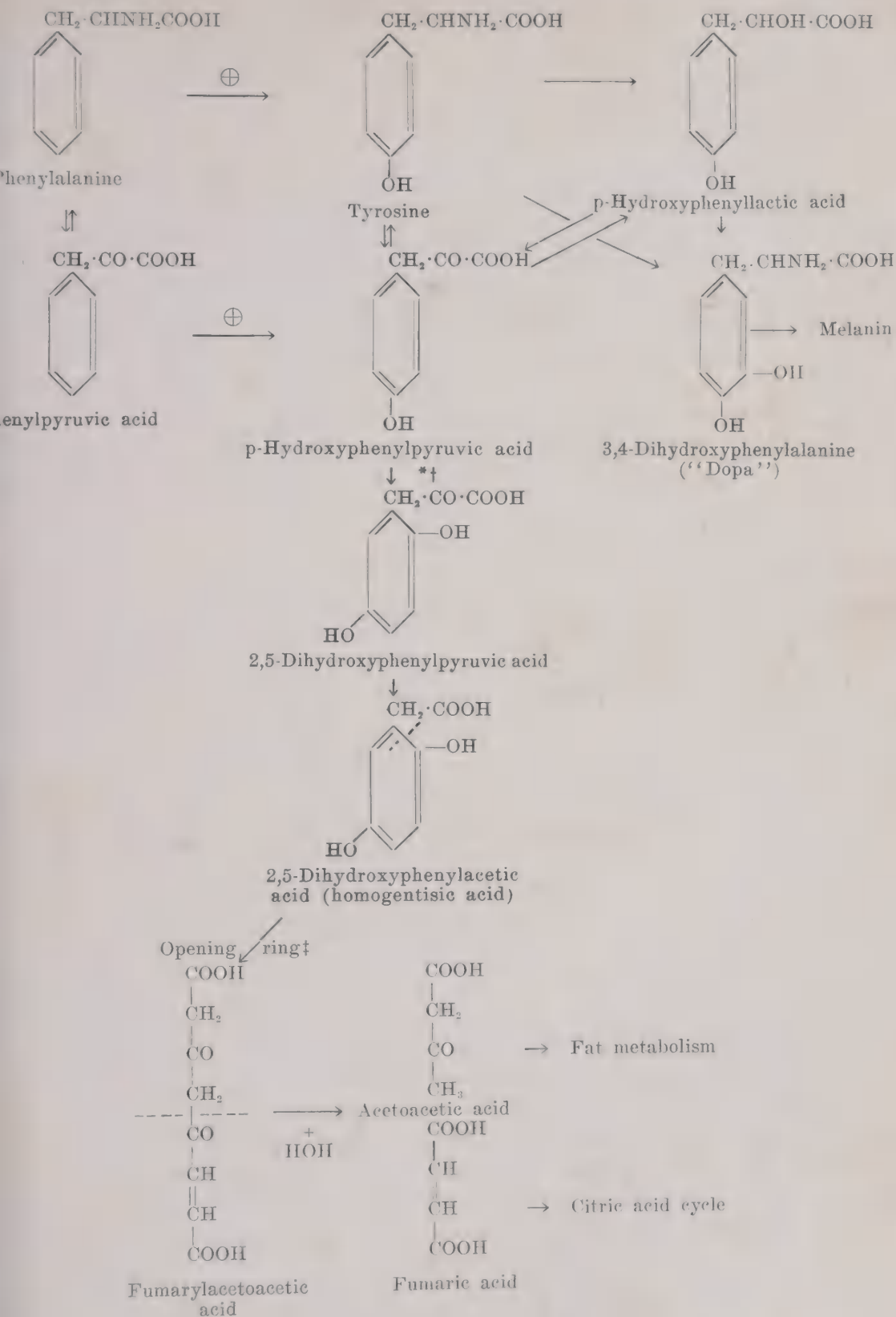
possible for the body. No doubt this is the reason why phenylalanine is an indispensable amino acid and tyrosine is not. That is, phenylalanine may be used by the organism, whenever either one is needed, whereas tyrosine cannot substitute for phenylalanine. Because of this relationship, these two compounds will be considered together.

By transamination tyrosine is converted to parahydroxyphenylpyruvic acid and phenylalanine to phenylpyruvic acid. The further transformations of these two have been deduced by studying two "inborn errors of metabolism." This term is applied to any condition of abnormal metabolism affecting an individual from birth. In *alcaptonuria* and in *tyrosinosis* there is an inability to metabolize completely phenylalanine and tyrosine. The former is a hereditary disorder, occurring more frequently in men than in women. Homogentisic acid is formed, is excreted in the urine, and, on exposure to the air, is oxidized to a blackish pigment which darkens the urine. This is a very rare condition, but still more uncommon is tyrosinosis. In the latter disease the aromatic amino acids are eliminated as tyrosine or as hydroxyphenylpyruvic acid.

If tyrosine is administered to patients with *alcaptonuria*, the excretion of homogentisic acid is increased. This probably indicates that homogentisic acid is intermediate in the normal metabolism of tyrosine, but that in this curious condition the body cannot carry the breakdown further. In homogentisic acid there are two hydroxyls, neither of which is in the para position to the acetic acid radical. How this rearrangement takes place is not known.

In tyrosinosis, Medes found that there were excreted in the urine p-hydroxyphenylpyruvic acid and its reduction product, p-hydroxyphenyllactic acid, and tyrosine and its oxidation product, 3,4-dihydroxyphenylalanine. Apparently there was difficulty in the early steps of tyrosine breakdown. That this was so can be seen from the fact that feeding homogentisic acid to the patient resulted in the complete utilization of this compound. In other words, in tyrosinosis the introduction of a second hydroxyl group and the shift of the two hydroxyls cannot be accomplished, but if such 2,5-dihydroxyphenyl derivatives are available, they are catabolized. In *alcaptonuria* the early steps can be brought about but not the last ones.

Normally phenylalanine is partly converted to tyrosine. This has been demonstrated repeatedly. Moss and Schoenheimer replaced a hydrogen of the benzene ring with deuterium and fed the labeled phenylalanine to rats. Labeled tyrosine was recovered from the tissue proteins. This reaction occurs in the liver of man, the enzyme requiring  $O_2$  as well as DPN or TPN (see page (405). Tyrosine is catabolized according to the middle vertical column of page 381 by another enzyme system in the liver. Transamination is responsible for the loss of  $NH_2$ ,  $\alpha$ -ketoglutarate being the acceptor. Vitamin C is a necessary factor. Phenylalanine is also partly oxidatively deaminized to phenylpyruvic acid, which is then converted to p-hydroxyphenylpyruvic acid. (Udenfriend and Cooper; Cammarata and Cohen; Knox and Le May-Knox.)



### Metabolism of Phenylalanine and Tyrosine

⊕ indicates points of blockage in phenylpyruvic oligophrenia.

\* indicates point of blockage in tyrosinosis.

† indicates point of blockage in vitamin C deficiency in human beings.

‡ indicates point of blockage in alcaptonuria and in vitamin C deficiency in guinea pigs.



Another intermediate appears to be 2,5-dihydroxyphenylpyruvic acid. Decarboxylation and oxidation yield 2,5-dihydroxyphenylacetic acid, known as homogentisic acid. The benzene ring is now opened, with the eventual formation of fumaric and acetoacetic acids. (Knox and Le May-Knox; Ravdin and Crandall.) Both phenylalanine and tyrosine are completely utilized by the normal body.

Another interesting anomaly of metabolism is connected with phenylalanine. Fölling in Norway first observed that certain feeble-minded children excrete a considerable amount of phenylpyruvic acid. This, as has been seen, is an intermediate in the breakdown of phenylalanine but not of tyrosine. Since these children are otherwise metabolically normal, they can apparently build phenylalanine into their body protein but cannot catabolize it in the normal manner. The biochemical error has been shown by Jervis to be an inability to introduce the hydroxyl group into the phenyl ring in the para position. Such patients can handle tyrosine, when fed, just as well as normal persons can. The condition is called phenylpyruvic oligophrenia.

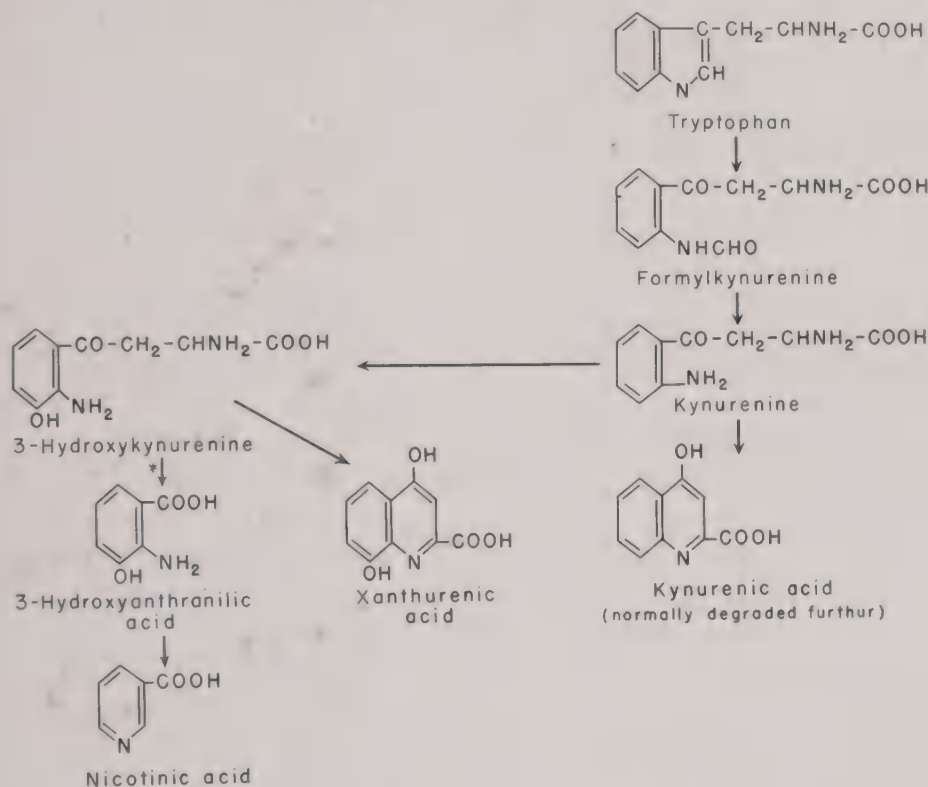
Experimentally, phenylpyruvic acid appears in the urine when extra phenylalanine is fed to rats having a thiamine deficiency. Thiamine, as cocarboxylase, may be essential to the decarboxylation of all keto acids arising in metabolism from whatever source. Vitamin C also is involved in some of these transformations. If tyrosine is fed to guinea pigs deficient in vitamin C, homogentisic acid is excreted in the urine (Seabock and Silberstein). Similarly, in prematurely born infants on vitamin C-deficient diets, tyrosine and phenylalanine are not metabolized in a normal manner, *p*-hydroxyphenyllactic acid and *p*-hydroxyphenylpyruvic acid being found in the urine. Since this is due to the absence of vitamin C, the administration of this vitamin restores metabolism to normal. ACTH (see page 627) has a similar effect, although it is not as effective as ascorbic acid, while the adrenocortical hormones have inconsistent or no action. (Levine.)

Another interesting phase in which tyrosine takes part is the production of certain pigments. Tyrosine is oxidized to dihydroxyphenylalanine, then to a red indole compound by an oxidizing enzyme, tyrosinase. This is subsequently converted to a "melanin." The melanins are brown or black pigments of ill-defined composition. They are deposited in the skin and hair and in the choroid coat of the eye, and their formation is aided by light; the production of freckles and tanning of the skin are common examples. In Addison's disease, there occurs a bronzing of those portions of the skin exposed to light. This indicates that the adrenal gland has some relation to the formation of melanins. Melanins are also deposited in melanotic sarcomas.

As will be seen, tyrosine constitutes an integral part of several hormones, as well as other compounds of biological importance. It is possible that it owes much of its activity to its reducing action. Along with the sulfhydryl compounds, tyrosine is a reducing agent of considerable importance and derives this property from its hydroxyl group. Tryptophan also has a reducing action.

it is far more rapid than has been generally thought, particularly in the presence of suitable buffers and at a pH and temperature approaching those found under physiological conditions.

**Tryptophan.**—The course of tryptophan catabolism is not entirely clear. In the mold *Neurospora* it can be synthesized from indole and serine, but it is questionable whether any such synthesis occurs in mammals, since tryptophan is an indispensable amino acid. Several intermediate compounds have been isolated in experiments on animals. Kynurenic acid, a related compound, is found in the urine of dogs, rabbits, and other animals. A study of the effects of feeding tryptophan upon the formation of kynurenic acid has thrown some



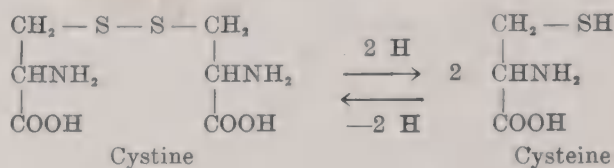
\*Point blocked by pyridoxine and riboflavin deficiencies (Henderson).

Fig. 46.—Paths of tryptophan metabolism.

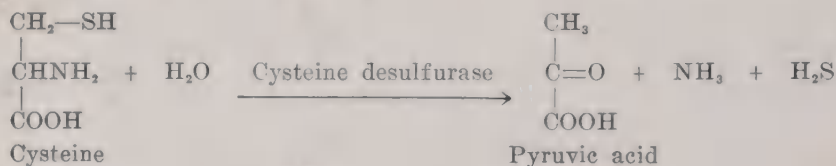
light upon the problem. Thus, when fed to rabbits, the amounts of kynurenine and kynurenic acid excreted are increased. Apparently the chief pathway of tryptophan metabolism is through kynurenic acid, which is completely decomposed by the organism. This is indicated by experiments in which several mammalian species were fed  $C^{14}$ -labeled tryptophan, resulting in the isolation of labeled kynurenine and kynurenic acid from the urine. (Heidelberger.) However, another path of metabolism is indicated in the biosynthesis of nicotinic acid from tryptophan. The amelioration of niacin avitaminosis may occur if the proteins of the diet are improved in their tryptophan content. (See page 291.) Consequently a partial source of this vitamin may be the production of it in the body from tryptophan. (Rosen.) The transformation

seems to be via kynurenine and 3-hydroxykynurenine. If pyridoxine is lacking in the diet, nicotinic acid is not formed, but xanthurenic acid results instead. Riboflavin also is needed for the nicotinic acid formation. (Axelrod; Porter; Mason.) (See Fig. 46.) It is interesting to note that patients suffering from, or threatened with, eclampsia also have a deranged type of tryptophan metabolism. If given a test dose of tryptophan, they excrete in the urine much larger amounts of xanthurenic acid than do normal pregnant or nonpregnant women under the same conditions. (Sprince.) An abbreviated scheme of the present ideas concerning tryptophan catabolism is given in Fig. 46. It will be noted that formation of kynurenine involves rupture of the pyrrole ring, with subsequent closure to form a new ring for kynurenic acid.

**Cystine and Cysteine.**—Since these two amino acids are readily interconvertible, it is probable that they undergo similar reactions in the body.



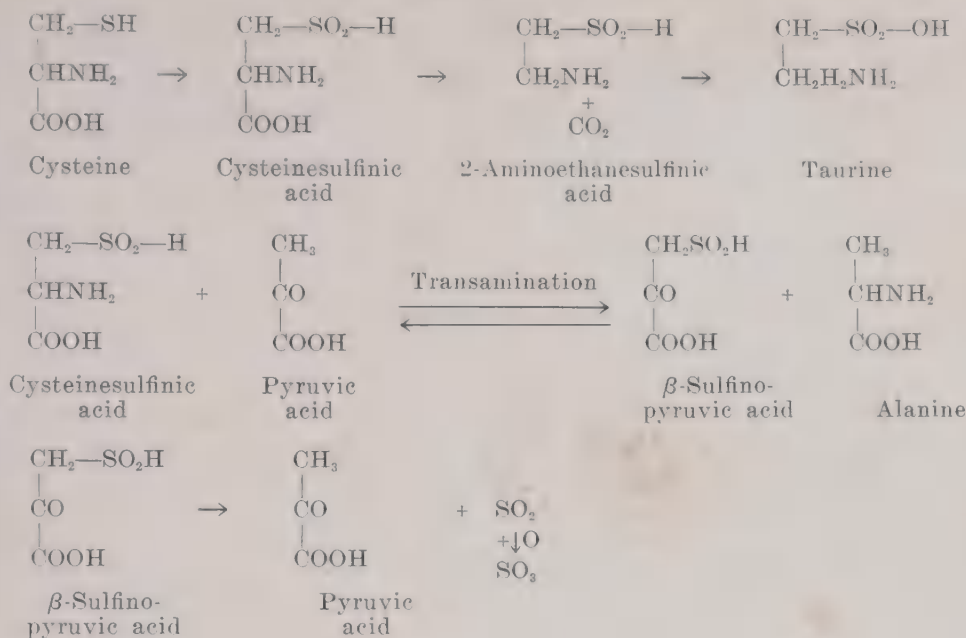
There is present in certain mammalian tissues an enzyme which rapidly converts cysteine to pyruvic acid, ammonia, and hydrogen sulfide. (Smythe.)



It is possible that this reaction can occur to cystine while it is in peptide linkage, but only when it is at either end of the peptide chain. (Greenstein.) The formation of pyruvic acid accounts for the fact that cystine and cysteine are among the amino acids convertible into glucose. The sulfur may be excreted as inorganic or organic sulfate or as part of the unoxidized sulfur of the urine. Cysteine may also be oxidized, in the rat, to cysteinesulfinic acid, which is then decarboxylated to form 2-aminoethanesulfinic acid, which has been named "hypotaurine." Hypotaurine may be oxidized to taurine, or cysteinesulfinic acid may undergo transamination with pyruvic acid, and eventually give rise to  $\text{SO}_3$  (Chatagner and Bergeret; Awapara; Awapara and Wingo). Thus it is seen how sulfates are formed from cysteine. A number of other reactions have been demonstrated by Fromageot. Some of them are shown on the following page. Instead of pyruvic acid,  $\alpha$ -ketoglutaric acid may enter into the transamination reaction, yielding glutamic acid. Besides taurine, other sulfur-containing compounds of physiological importance are also probably derived from cysteine or cystine. Among these are insulin, glutathione, and ergothioneine.

Cystine occurs in the urine of certain individuals. It is another "inborn error of metabolism," and is hereditary. Persons suffering from this abnormal



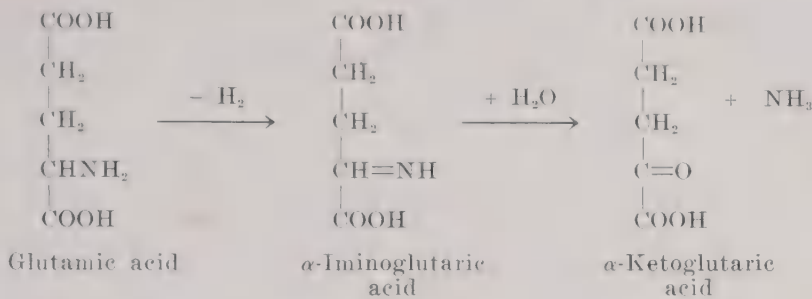


condition excrete this amino acid even on a protein-free diet. If cystine itself is administered, they do not excrete it. This is also true of homocystine, the next higher homologue, and of glutathione. However, cysteine, homocysteine, and methionine are excreted largely as extra cystine in the urine. The explanation of these facts is, perhaps, as follows: In cystinuria the body cannot oxidize cysteine to cystine. The cysteine therefore is carried to the kidneys. Here, however, oxidation to cystine does occur and this compound is excreted or deposited as cystine crystals or cystine calculi. When cystine is fed, since it is already oxidized to a state which this organism can handle, it can now be oxidized to sulfate. It has recently been found that cystinurics also excrete arginine, lysine, ornithine, and leucine in amounts far greater than normal. (Stein.) Whether or not this is related to the same enzyme deficiency, which is responsible for the failure to oxidize cysteine is not known.

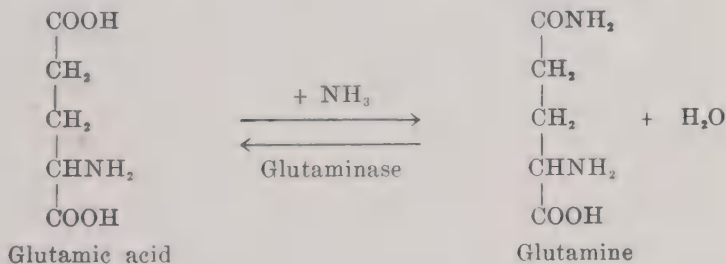
**Methionine.**—Methionine can undergo oxidative deamination, or perhaps transamination, with the formation of the corresponding keto acid and ammonia. The subsequent fate of this residue is not known. There are, however, other metabolic pathways which are very interesting. Methionine is an indispensable amino acid, while cystine is not, and the former evidently can be changed into the latter, but cystine cannot be transformed into methionine. When methionine, containing isotopic sulfur,  $\text{S}^{35}$ , was fed to animals, the isotopic sulfur was isolated from the tissue proteins. By similar experiments the pathway was indicated to be: methionine to homocysteine, with the loss of a methyl group. The methyl group may be oxidized to carbon dioxide and water or may enter transmethylation reactions. (See page 390.) Homocysteine then may be oxidized to homocystine or be converted to cysteine, after having been coupled with serine to yield cystathionine as an intermediate product. The cysteine then may be oxidized to cystine. The further catabolic pathway of cystine is



to succinic acid anaerobically, but by oxidative deamination it is transformed to  $\alpha$ -ketoglutaric acid. (von Euler; Olson and Anfanger.)

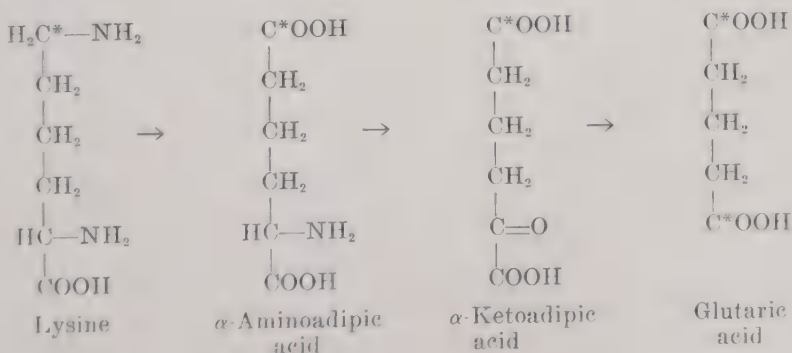


Transamination (see page 366) also converts glutamic acid into  $\alpha$ -ketoglutaric acid and aspartic acid into oxaloacetic acid. Both of these amino acids yield glucose in diabetic animals and in each case three carbons are so utilized. Both bind ammonia, forming acid amides, and because of this are of importance in ammonia transport, and also in ammonia formation.



Glutaminase, the enzyme which can effect this reaction, is present in kidney, brain, and retina. The corresponding acid amide of aspartic acid is asparagine.

**Lysine.**—This diaminomono-carboxylic acid is in a class by itself. It is the only amino acid which, when once present in tissues, does not exchange its nitrogen with other amino acids circulating in the body fluids. When lysine is fed, however, it can give up its nitrogen to those present in the tissues just as the others do, but if it has lost its nitrogen, it cannot be reaminated. Since it can contribute its nitrogen in the direction just mentioned, it is evident that it is deaminated but just how it is deaminated is not known. The nonnitrogenous residue does not yield either glucose or acetoacetic acid. The metabolic route may be the following:

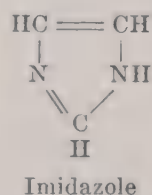
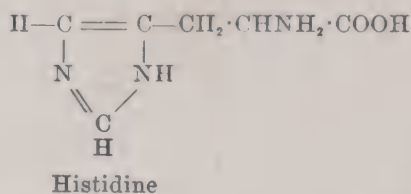




Using lysine containing an  $\epsilon$ -labeled carbon, Borsook and his group found that guinea pig liver converted it in vitro to  $\alpha$ -amino adipic acid. When the latter, similarly labeled, was the starting point, it was oxidatively deaminated to  $\alpha$ -keto adipic acid, which was then oxidatively decarboxylated to glutaric acid.

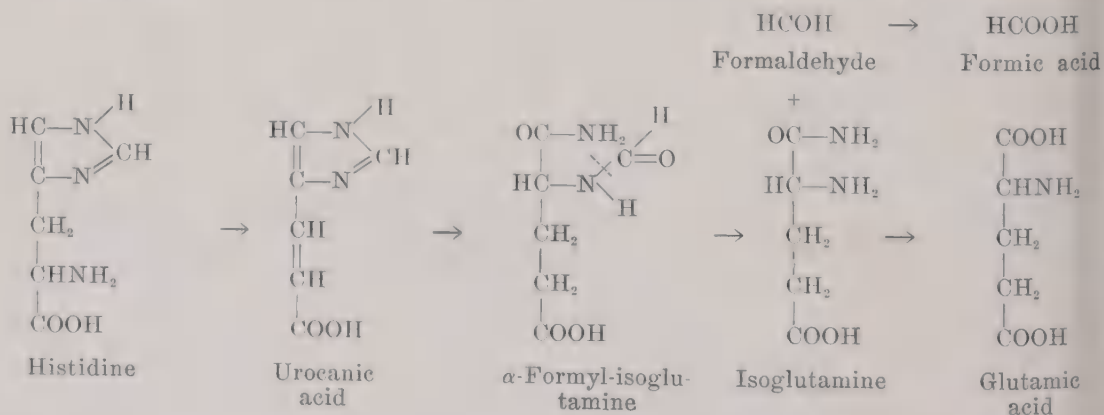
**Arginine.**—It has been seen how arginine takes part in the formation of urea, yielding ornithine. It is unlikely that all of the ornithine thus formed continues in that cycle. It may be converted to proline (see page 389). Another suggestion is that it yields succinic acid. At any rate, both arginine and ornithine yield glucose in the diabetic animal, and succinic acid logically should be an intermediate. Arginine, as will be seen, also contributes to the synthesis of creatine.

**Histidine.**—Histidine is a most interesting compound and furnishes a number of problems. Its metabolic fate is by no means settled. When fed or injected, only traces of it are excreted, and yet when imidazole itself is administered most of it is eliminated.



An enzyme, histidase, which opens up the imidazole ring only in histidine, but not in imidazole, is found in the liver and other organs.

Since glutamic acid, ammonia, and formic acid are formed as final products of histidase activity, and since urocanic acid has been found in the urine after injection of large quantities of histidine, the path of metabolism of this amino acid may be the following: (Sera and Yada; Tabor and Hayaishi; Abrams and Borsook):



The L-amino acid oxidase of rat liver and kidney also attacks histidine with the formation of the keto acid and ammonia.

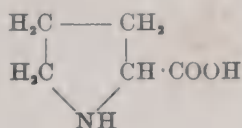
During pregnancy large amounts of histidine are excreted in the urine. This occurs from about the fifth week of pregnancy until a few days postpartum. The absence of histidase from the liver during this period accounts

or this phenomenon, and the explanation given is that nature thus provides the fetus with a superabundance of this indispensable amino acid.

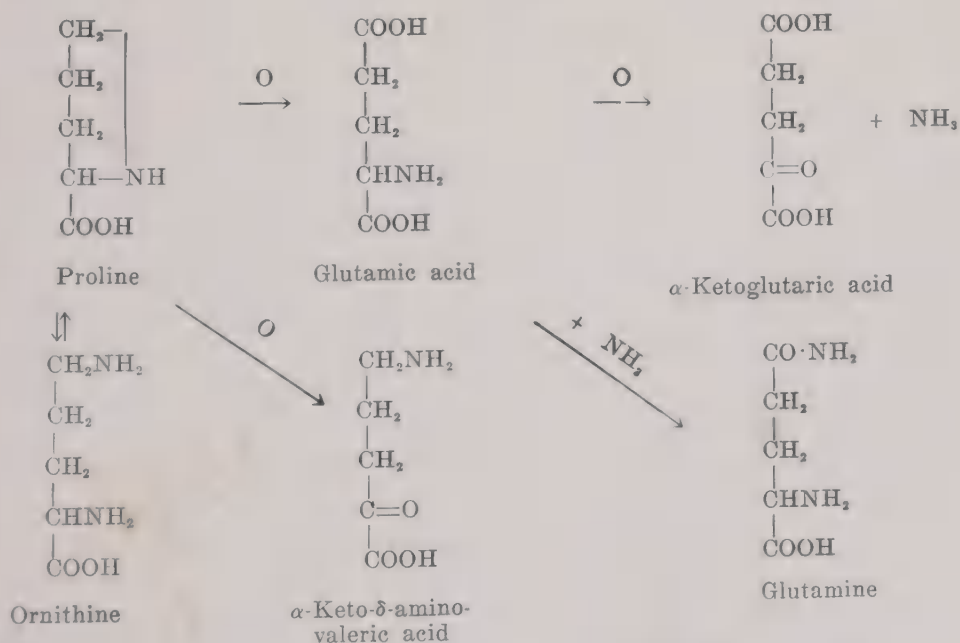
The formation of histamine from histidine by intestinal bacteria has been described (page 250). It is also formed in certain tissues by the action of histidine carboxylase. Histamine has a vasodilating action, thereby lowering blood pressure, and also is a powerful stimulant to gastric secretion. Consequently the regulation of its concentration is quite essential. Histamine is destroyed by histaminase, an enzyme occurring in most tissues. A notable exception is the lungs and, consequently, large quantities of it accumulate there.

Two dipeptide derivatives of histidine are found in muscle. Carnosine is beta-alanyl histidine and anserine is beta-alanyl-methyl histidine. Their roles are not understood.

**Proline.**—Malherbe and Krebs, after incubating kidney tissue with proline, found alpha-ketoglutaric acid, ammonia, and a substance believed to be glutamine. If proline



is written with its four carbons in one line, we can see the close relationship to these compounds and understand how glutamic acid can be the intermediate compound formed.



Other evidence in favor of this is the fact that both proline and glutamic acid are glucogenic.

Hydroxyproline may be catabolized by the same route, but if it is, it is only to a slight extent, because hydroxyproline is not a glucose former. On the contrary, it gives rise to acetoacetic acid in the diabetic organism.

The proline ring can also be opened by the  $\alpha$ -amino acid oxidase previously mentioned. In this case  $\alpha$ -keto- $\delta$ -aminovaleric acid is produced. This method of handling proline is also shown on the scheme (above).

Proline and ornithine are readily converted into each other in the body. (Stetten and Schoenheimer; Shemin and Rittenberg, 1945.) Thus, another metabolic route is seen; that is, proline may yield ornithine for urea synthesis, or ornithine may be broken down via proline.

**Transmethylation.**—The discovery of the transfer of methyl groups from one compound to another is one of the recent great advances in biochemistry. We owe this mostly to du Vigneaud and co-workers. These relationships have a bearing on fat metabolism, sulfur metabolism, and creatine metabolism. At the moment, of particular concern are sulfur and creatine metabolism.

The occurrence of methylated compounds has long been known, as well as the recognition of their peculiar importance in physiology. In fact, Hofmeister in 1894 proposed a hypothesis which has now been largely substantiated. He suggested that the body is unable to manufacture methyl groups and consequently must obtain them in the diet. As will be seen, this is not quite true, since methyl groups can be synthesized by the organism if conditions are favorable. Once absorbed, the methyl group may be transferred from one compound to another as a unit but only to and from certain compounds and not indiscriminately. This does not depend upon the methyl group, which has but one form, but upon the entire molecule. Certain compounds are methyl donors, the chief of which are choline, methionine, and betaine. The "labile" methyl is attached either to a nitrogen or a sulfur atom in their molecules. The fact that a methyl group is removable from a compound does not necessarily mean that it is "labile"; i.e., transferable to another compound. Sarcosine,  $\text{CH}_2\text{NH}(\text{CH}_3)\text{COOH}$ , may lose its methyl group in the body, but this does not seem to be available for transmethylation reactions except to a very slight extent.

The starting point is the fact that although methionine is an indispensable amino acid, young animals will grow on a methionine-free diet, containing homocysteine, provided choline or betaine is also present. Choline and betaine both contain methyl groups, and the explanation is that their methyl groups are labile and are easily transferable to homocysteine, to change this amino acid to methionine. Since homocysteine alone would not permit growth, it was inferred that the animal organism is incapable of generating methyl groups for this methylation. By providing the suitable constituents, the "indispensable" amino acid is synthesized by the body. This was later substantiated by isotope experiments. If choline, containing deuterium in the methyl group, was fed to animals along with homocysteine, the methionine isolated from the proteins of the animal's tissues was found to contain deuterium.

The reverse process also occurs. This was proved by feeding methionine containing deuterium in the methyl group to animals. On a diet free from choline and ordinary methionine, the administration of "deuteriomethionine" led to the



discovery of choline containing deuterium in the tissues. The methyl group had been used to form new choline. Furthermore, some had also been used to form creatine. (See page 396.) The demethylation of methionine is, therefore, a reversible reaction. A similar experiment has been performed on man, thus showing that transmethylation reactions also take place in the human organism (Simmonds and du Vigneaud).

Since the methyl group of methionine can be used to form creatine, the possibility was suggested that choline's methyl group might similarly be used. Isotope experiments proved that this could occur. "Deuteriocholine" given to animals on an otherwise choline-free, methionine-free diet was found to result in the presence of creatinine containing deuterium in the urine.

In transmethyations the substance yielding the methyl group is called the methyl donor and the one receiving it is the methyl acceptor. For the synthesis of choline, ethanolamine is considered the methyl acceptor. Feeding of this compound, labelled with  $N^{15}$ , resulted in the formation of choline containing  $N^{15}$ . (Stetten.) In the synthesis of methionine, homocysteine is the methyl acceptor, and in creatine synthesis glycocyamine (guanidoacetic acid) functions in the same way. Creatine does not become a source of methyl groups when it is present in the diet or when it is formed in the course of a transmethylation.

The labile methyl group can also be synthesized by the animal. This has been demonstrated by giving animals  $D_2O$  in their drinking water and finding deuterium in some of the choline of their tissues. (du Vigneaud, 1950.) Since the animals were raised in the absence of bacteria, synthesis by microorganisms was ruled out. Vitamin  $B_{12}$  and folic acid are involved in this synthesis, and it is quite probable that the absence of a sufficient quantity of these vitamins in the diet was responsible for the inability to achieve the biosynthesis of methyl groups in the work previously mentioned. However, very young animals do not possess this capability, and the biologically labile methyl groups are placed in a position analogous to that which arginine holds among the essential amino acids. They can be synthesized but not at a rate rapid enough for the welfare of the young animal.

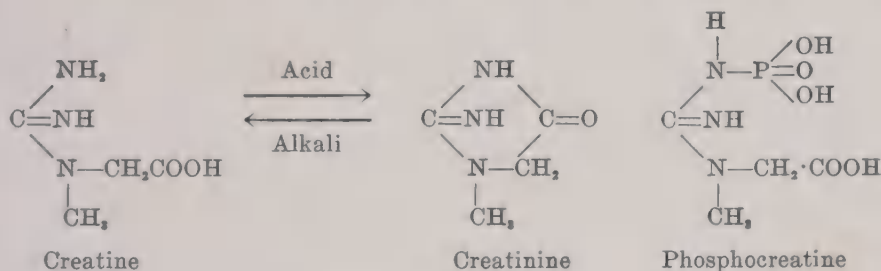
The labile methyl group may be produced by the body from a variety of compounds. Following the discovery by Sakami, that  $C^{14}$ -methyl-labeled acetone, after administration to rats, resulted in the  $C^{14}$  being placed as a  $\beta$ -carbon of serine, the reverse reaction was attempted by Arnstein. He found that serine carrying a tagged  $\beta$ -carbon yielded choline bearing tagged  $CH_3$  groups. Other effective precursors are formaldehyde, formate, methanol, and the  $\alpha$ -carbon of glycine. In other experiments it was discovered that acetone, creatosine, histidine, and tryptophan, in their metabolic reactions, either yield methyl groups directly or one-carbon units which are easily converted into methyl groups. Methyl groups from these sources, or from choline or betaine, may be transferred to form methionine. It is possible that choline acts only indirectly as a methyl donor, in that it must first be oxidized to betaine.



Besides its role as promoter of growth in the presence of homocysteine and absence of methionine, the labile methyl group has other effects. It has a lipotropic action; that is, it inhibits fatty liver formation under certain circumstances. It prevents perosis, or "slipped tendon disease," in chicks and hemorrhagic degeneration of various organs. Thus labile methyl groups resemble choline in function (see page 304) and, of course, the reason is obvious—choline is an important source of labile methyl groups. The labile methyl group of methionine can be oxidized to  $\text{CO}_2$ . Methionine, containing  $\text{C}^{14}$  in the methyl group, was fed to a rat with the result that some of the expired  $\text{CO}_2$  contained  $^{14}\text{C}$ . (Mackenzie, 1947.) It is probable that excess labile methyl groups from this and other methyl donors are taken care of in this way.

### CREATINE AND CREATININE

Creatine is methyl guanidine acetic acid and creatinine is its anhydride. Their close relationship and the formula for phosphocreatine are shown below.



The conversion of creatine to creatinine by acid is a quantitative reaction and is the method used when estimating this substance, since creatinine is easily determined. The reverse reaction is not quantitative. Phosphocreatine, or phosphagen, is a creatine derivative containing labile, or energy-rich, phosphate and yields energy during muscle contraction. The physiological relations between creatine and creatinine have puzzled biochemists for many years and although many facts concerning them are now known, there is still a great deal which is obscure. About 120 Gm. of creatine and phosphocreatine is present in the human body, mostly in the muscles. Very little creatinine is found there. Neither creatine nor creatinine has been found among the hydrolysis products of any protein. Consequently their origin presents a problem. A small part of it has just been considered under transmethylation; i.e., the origin of the methyl group. We should, however, inquire into the normal and pathological occurrence of both compounds and their behavior when administered, as well as any other available pertinent facts.

Since creatine is present in muscle tissue, and is water soluble, it will be found in meat, meat gravies, meat soups, and meat extracts. Phosphocreatine is easily hydrolyzed to creatine and phosphoric acid that no phosphocreatine is such is present in our food. Children regularly eliminate creatine in their urine in larger amounts than do adults. It was formerly believed that normal adult males excrete no creatine in their urine but that normal adult women



excrete moderate amounts at irregular intervals. This has been the subject of controversy, but now, according to Wilder and Morgulis, creatine must be recognized as a normal component of the urine of healthy men. It constitutes about 6 per cent of the total creatine-creatinine output; i.e., about 60-150 mg. per day. Most women excrete about twice as much as men, and much more irregularly. In about 20 per cent of females the excretion of creatine does not exceed that of the male. During pregnancy the output of creatine increases, and for two or three weeks *post partum* it is found in even greater amounts than previously. The probable explanation for most of these facts is that the reaction



takes place in the muscles and does so only when the muscles are functioning efficiently. The adult musculature is ordinarily more efficient than that of the child. In pregnancy, a large amount of uterine muscular tissue is formed and is not functioning; and *post partum* the reduction of this tissue may release creatine stored up there. However, the same increased creatine output occurs after cesarean section and removal of the uterus. The output of creatine is also greater in starvation, carbohydrate deprivations, diabetes, hyperthyroidism, fevers, and malnutrition. In all of these conditions there is an increased catabolism either of muscular tissue or of tissue proteins in general. Diseases peculiar to the musculature frequently are accompanied by creatinuria. In myasthenia gravis it is not always found, and in myotonia congenita, it is seldom found, but in dystrophia myotonia (progressive muscular dystrophy) and in amyotonia congenita, creatine invariably appears in the urine. Creatine elimination is greatly increased in rheumatoid arthritis but not in osteoarthritis. (Granirer.)

The relationship of creatine to creatinine was investigated at an early date by feeding creatine to animals. The first trials seemed to indicate that creatine was not converted to creatinine in the body. Rose and Dimmitt fed doses of 10 or 20 Gm. of creatine to men and found increases in urinary creatinine of only about 0.2 to 0.5 Gm., the bulk of the creatine being eliminated unchanged. Administration of creatinine, however, did not lead to a formation of creatine, and most of the creatinine was excreted. No urea was formed from either compound. It is evident that the reaction

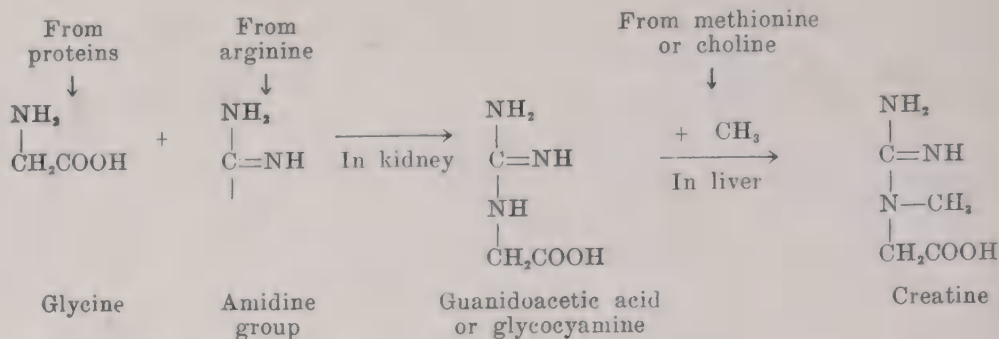


in the body is not reversible. This was corroborated by Benedict and Osterberg who fed a dog small amounts of creatine daily for seventy days. Appreciable amounts of creatine began to be excreted after ten days, and "extra" creatinine appeared after a week of creatine feeding. The creatinuria occurred as long as the feeding continued, but the increased elimination of creatinine persisted much longer. Almost half of the creatine fed could not be accounted for. The experiment therefore indicates that although creatine is converted to creatinine in the body, this is not the only pathway for its catabolism. Isotope experiments have given more direct evidence of this relationship. Bloch and Schoenheimer

fed small amounts of creatine containing  $N^{15}$  to adult rats. This  $N^{15}$  was found to be present in the creatine of muscle and internal organs, as well as in urinary creatinine. In a second series, the tissue creatine was tagged with isotopic nitrogen by feeding isotopic creatine during a preliminary period. Then, when creatine feeding was discontinued, the isotopic content of the urinary creatinine was identical with that of the body creatine. These facts indicate (1) that creatine in the diet can be absorbed and replace the creatine of the tissues and (2) that, on a creatine-free diet, the tissue creatine is the sole source of urinary creatinine. When isotopic creatinine was fed, however, no isotopic nitrogen was found in the tissue creatine, again emphasizing the fact that the reaction, creatine  $\rightarrow$  creatinine, is biologically irreversible.

Creatinine is always present in the urine. It is an end product of creatine metabolism. The daily output on a creatine-creatinine free diet is almost constant for a given individual, and the amount in milligrams excreted per day per kilogram of body weight is called the creatinine coefficient. The creatine present in the urine should be added to the creatinine. For most normal men the creatinine coefficient will vary between 18 and 32 with an average of about 25, and in women the normal range is between 9 and 26 with an average of about 18. Children have lower values. In general, the better the muscular development, the higher the creatinine coefficient. Consequently obese individuals are likely to have low coefficients because much of their weight is not muscle. This indicates that every individual has a characteristic creatine-creatinine turnover dependent, in a general way, on the amount of functioning muscle *tissue* but independent of the degree of muscular *activity*. Lindquist has found that when phosphocreatine is hydrolyzed in vitro at  $38^{\circ}\text{C}$ ., about 10 per cent is converted into creatinine. This led him to surmise that in the living organism a part of the phosphocreatine similarly spontaneously goes to form creatinine; hence the fact that the daily output of creatinine varies with the *total amount of musculature*. The reaction of phosphocreatine, resulting in the transfer of energy-rich phosphate to some acceptor, does not yield creatinine, and this harmonizes with the fact that the creatinine output is not related to muscular activity.

The creatine content of the body is derived, in part, from creatine in the diet. Any creatinine in the diet, of course, cannot be utilized as such. However, as has been said, on a creatine-creatinine free diet there is a constant output of creatinine. This indicates a synthesis of creatine by the body. How does this arise? We cannot even touch on the many earlier experiments in this field. Suffice it to say that Bloch and Schoenheimer by isotopic experiments have very beautifully elucidated the mechanism, although it must be said that many investigators had previously brought evidence tending in the same direction. Feeding a number of possible precursors containing  $N^{15}$ , they found that arginine and glycine are the only natural amino acids investigated so far which are precursors, to any considerable extent, of creatine. Each of these two supplies nitrogen to different parts of the creatine molecule, as the scheme below shows. As has been seen, the methyl group may be derived from either methionine, choline, or betaine.



Very small amounts of isotopic creatine were found to arise after feeding isotopic ammonia, leucine, tyrosine, and glutamic acid. Apparently these are indirect creatine precursors, since their nitrogen is transferred to the amidine group of the arginine of proteins, which in turn is shifted to glycine to form glycoeyamine.

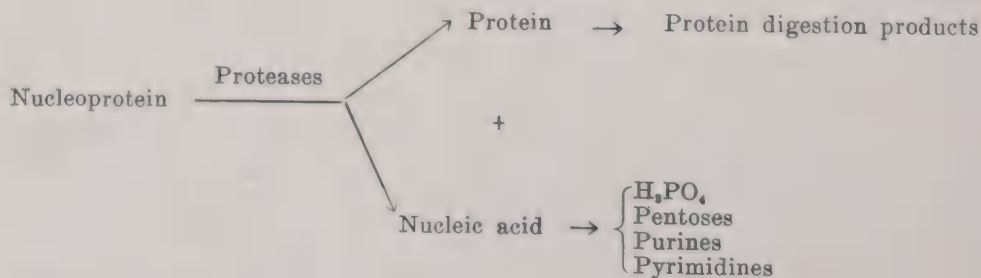
To summarize creatine and creatinine metabolism :

1. Creatine is an essential physiological constituent of the body. Under conditions of maximum muscular efficiency it is not excreted as such.
2. Creatine is converted into creatinine physiologically. The amount of creatinine excreted is fairly constant and bears some relation to total musculature.
3. Tissue creatine may be obtained from food creatine but is largely synthesized. Glycine and a fraction of arginine unite to form glycoeyamine; then methyl groups are added to complete the synthesis.
4. Creatinine is a waste product and is not converted to creatine.

## PURINE AND PYRIMIDINE METABOLISM

Since the purines and pyrimidines enter the body chiefly as constituents of nucleic acids, and occur in the body mainly in that form, this discussion is in a large sense a study of nucleic acid metabolism. In fact, purine metabolism parallels that of the nucleic acids so closely that "purine metabolism" and "nucleic acid metabolism" are almost synonymous.

Nucleoproteins are conjugated proteins composed of one or more protein molecules united with nucleic acid. When they are ingested in food they undergo the following disintegrations :



The nucleic acids are, accordingly, composed of phosphoric acid, pentoses, purines, and pyrimidines. The protein component varies with the species as



cell as with the tissue in which it occurs and may yield a considerable amount of the basic amino acids on hydrolysis. The nucleic acids, however, seem to be of two main varieties, ribose nucleic acid and desoxyribose nucleic acid, originally called yeast nucleic acid and thymus nucleic acid, respectively. Although it was formerly thought that the former occurred only in plant tissues and the latter only in animals, this has been shown to be incorrect. The two nucleic acids differ in their pentose constituents and in part of their pyrimidine constituents, as shown as follows:

## HYDROLYTIC PRODUCTS OF

## RIBOSE NUCLEIC ACID

Phosphoric acid

D-Ribose

Pentoses

Adenine

Guanine

Purines

Cytosine

Uracil

Pyrimidines

## DESOXYRIBOSE NUCLEIC ACID

Phosphoric acid

D-2-Desoxyribose

Adenine

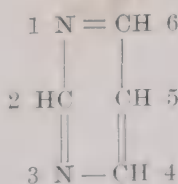
Guanine

Cytosine

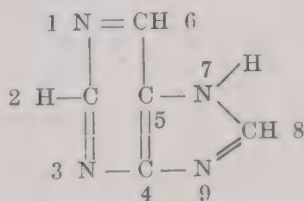
Thymine

Desoxyribose nucleic acid is found in the chromatin of the cell nucleus, whereas ribose nucleic acid is present in cell cytoplasm and also in the plasmosome (nucleolus). They may easily be distinguished chemically. One test, the Feulgen test, consists of a mild hydrolysis with HCl and subsequent treatment with reduced fuchsin. If desoxyribose is the sugar split off, it will produce a red color with the dye, whereas ribose will not. Another pyrimidine, 5-methylcytosine, has been found in small quantities in some nucleic acids, and it is quite possible that other purines, pyrimidines, or pentoses will be discovered as research continues.

**Structure of the Purines and the Pyrimidines.**—At this point the structural formulas for the pyrimidines and purines of the nucleic acids, as well as for the other physiologically important purines, will be given. The pyrimidine and purine nuclei are first given, with the nitrogen and carbon atoms numbered for convenience in designating where various groups or atoms are placed.



Pyrimidine

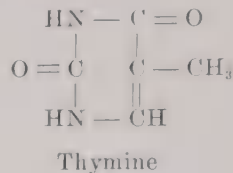
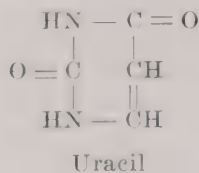
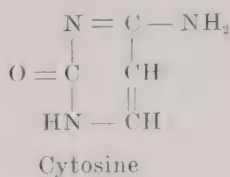


Purine

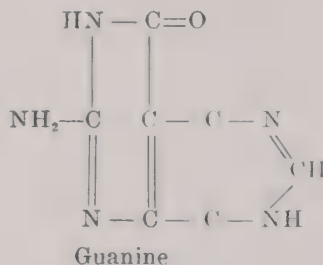
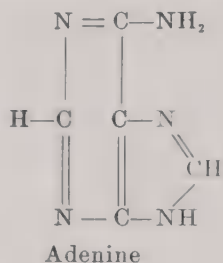
It will be noted that the pyrimidine ring forms a part of the purine ring (1 to 5). This is of interest in view of the fact that both types of substance constitute parts of the nucleic acid molecule.

\*According to a recently suggested system of numbering the pyrimidines, nitrogen 3 is designated as nitrogen 1, and numbering is in a clockwise manner. The new numbering system was used in the third edition of this textbook, but since it has not been generally adopted in biochemical literature, the older system is again being given.

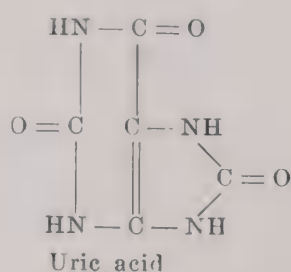
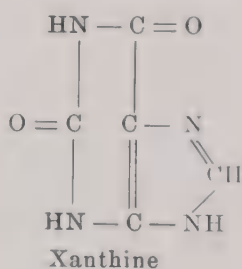
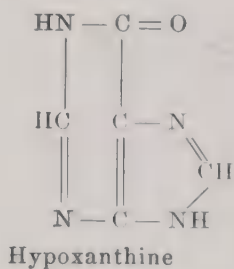
The pyrimidines of the nucleic acids are cytosine (6-amino-2-oxypyrimidine), uracil (2,6-dioxypyrimidine), and thymine (2,6-dioxy-5-methylpyrimidine, or 5-methyl uracil).



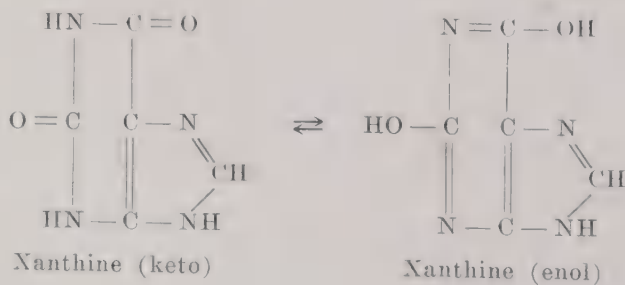
Both of the purines, adenine and guanine, are found in all nucleic acids. Adenine is 6-aminopurine, and guanine is 2-amino-6-oxypurine.



The other purines, of prime importance physiologically, are hypoxanthine, xanthine, and uric acid. Hypoxanthine is 6-oxypurine, xanthine is 2,6-dioxypurine, and uric acid is 2,6,8-trioxypurine.

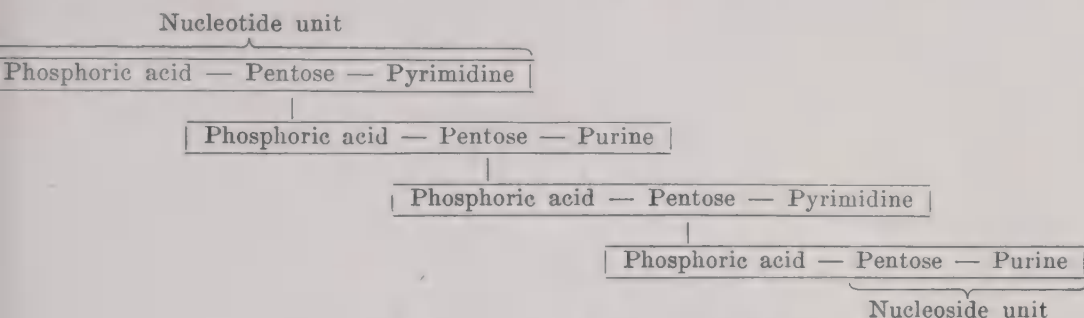


The oxypurines may also be written in the enol forms, which are in equilibrium with the keto forms; thus:

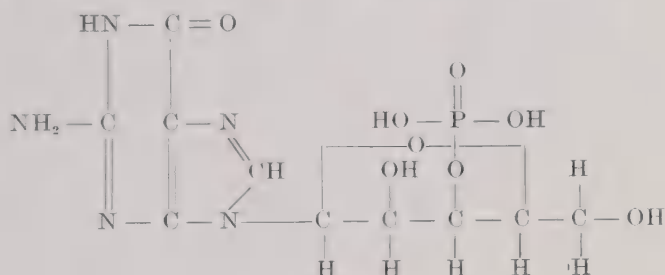


**Structure of the Nucleic Acids.**—Appropriate enzymes can split nucleic acid into four different “nucleotide” units. Each nucleotide is the phosphoric acid ester of a “nucleoside,” and each nucleoside is a pentose derivative of one of

the previously listed purines or pyrimidines.\* Diagrammatically, a tetranucleotide, which was long thought to be the structure of the nucleic acids, is as follows:



In the purine nucleotides the pentose is joined to the nitrogen at position 9 of the purine and the phosphate is usually on carbon 3 of ribose, or carbon 5 in the case of deoxyribose. Thus a typical purine nucleotide is guanine-ribose-nucleotide, or guanylic acid, with the formula:

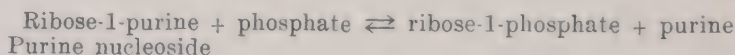




The molecular weight of a typical "tetra-nucleotide" is 1,286. Using diffusion, ultracentrifugation, and other methods, it has been found that the molecular weights of nucleic acids vary from once or twice 1,286 up to 1,000,000 or more, depending upon the source and the method of isolation of the preparation. It is therefore probable that the nucleic acids are highly polymerized tetra-nucleotides. The exact method whereby the units are linked together is not known. (Tipson.)

The earlier work, which led to the proposal of the above formula, was based largely on the supposed equimolecular distribution of the four purine and pyrimidine bases. Modern techniques have demonstrated that this is not always the case, and therefore the tetranucleotide formula shown above may not be the correct one. (Schlenk.) For example, there is evidence to indicate that the ribosenucleic acid, derived from yeast, may consist of long chains of purine nucleotides linked to long chains of pyrimidine nucleotides. (Schmidt.) It is even possible that the two general types of nucleic acids may be quite different in architecture.

After nucleic acid is liberated from food nucleoprotein in the intestinal tract by proteases, it is depolymerized and split into its constituent nucleotides by a nucleinase found in the small intestine. The nucleotides are attacked by nucleotidases, yielding phosphoric acid and purine nucleosides or pyrimidine nucleosides. The nucleotidases are phosphatases, which are not specific for these particular substrates. Nucleosidases split the nucleosides into their purine or pyrimidine and carbohydrate components. Kalekar has presented evidence that the nucleosidase reaction is not hydrolytic but phosphorolytic; thus:

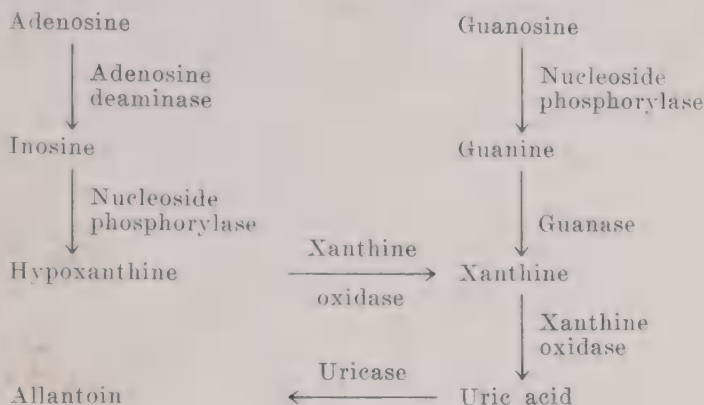


These enzymes are more correctly termed "nucleoside phosphorylases." All of the enzymes mentioned are present either in intestinal juice or in the intestinal mucosa. The result, however, is the breaking down of the nucleic acids into their constituents—pentoses, phosphoric acid, purines, and pyrimidines—in the small intestine. It is quite probable that the nucleoproteins of the cells break down to some extent in a similar way, but of this we are not sure. If so, purines and pyrimidines would be released and would be subjected to the same reactive agents as those derived from foods.

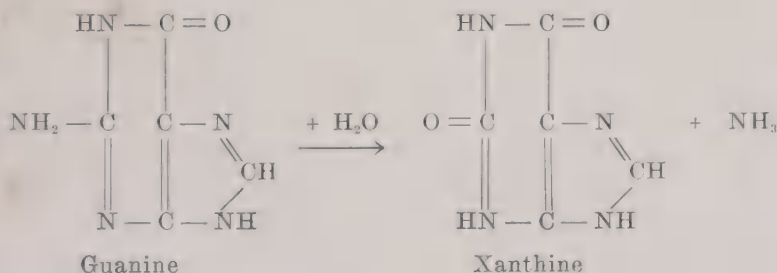
The adenine and guanine, formed from nucleic acid digestion, are absorbed and the adenine is utilized, in part, for the synthesis of nucleic acids in the tissues. This has been demonstrated by feeding this purine, labeled with isotopic nitrogen in its pyrimidine ring, to rats and isolating the nucleic acids and other related products after a suitable interval. Some of the adenine was converted to guanine. Labeled dietary guanine was not incorporated into body nucleic acid to the same extent that adenine was. Apparently nucleic acid guanine arises in intermediary metabolism. (Brown.) In fact, it has been shown that the body does not require preformed purines or pyrimidines in the diet for the production of tissue nucleotides.

The next problem is the fate of adenine and guanine. Although an adenase is present in low concentration in the tissues of some mammals, it is

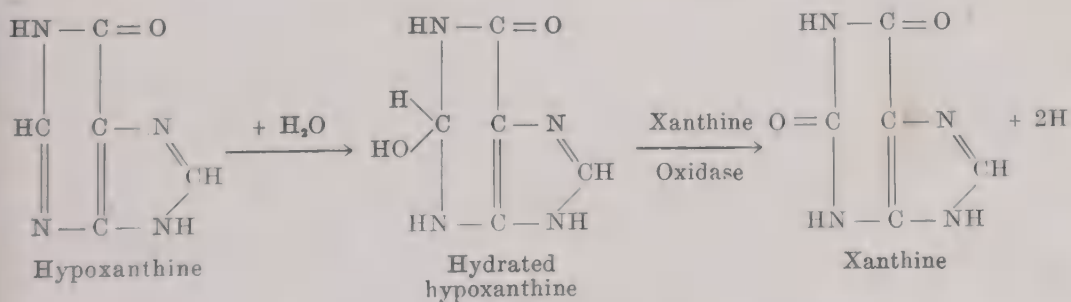
absent in man. Since adenine is not excreted, Rose has suggested that it is probably deaminized while still combined as a nucleoside. The following pathway has been suggested by Christman:



Guanase is a deaminase which catalyzes the conversion of guanine to xanthine by hydrolytic deamination. The reaction is shown below.



Xanthine oxidase is one of the enzymes containing riboflavin. It is a dehydrogenase and oxidizes by transferring hydrogen to a hydrogen acceptor. The reaction with hypoxanthine is as follows. The same enzyme oxidizes xanthine to uric acid in an analogous manner, and uric acid is oxidized by uricase to allantoin.



Allantoin is an end product found in the urine of many animals but not in that of man or the anthropoid ape.

On a diet rich in nucleoproteins or purines the uric acid content of the urine is much higher than ordinarily. Such a diet is one which includes meat, particularly liver and other glandular meats, meat extracts, and certain vegetables, such as legumes, mushrooms, and spinach. The conversion of dietary

purines to uric acid is not quantitative, however. Probably there are other catabolic routes for the purines than those now known. This problem has recently been studied with the aid of isotopically marked uric acid. If such uric acid is injected into a person, and the concentrations of total and tagged uric acid in the blood are followed for several days, a number of interesting facts are brought out. For example, it was calculated that the uric acid synthesized per day was about 150 mg. more than the amount excreted, indicating that this amount had been destroyed. More recent experiments showed that about 78 per cent of injected uric acid was excreted unchanged in the urine. Of the remainder, most was degraded to urea, a small amount to ammonia, and some was excreted in the feces. (Wyngaarden and Stetten.) The normal "uric acid miscible pool," i.e., the total uric acid with which the isotopic uric acid can mix, amounts to about 1200 mg. In gout this is increased three to twenty-five times, an amount undoubtedly greater than could be in aqueous solution in the volume of body fluid available. It is therefore concluded that the outer layers of the gouty uric acid deposits, or "tophi," are a part of this pool. It is suggested that this large increase in uric acid in gout is a result of increased purine synthesis. (Stetten.)

The urinary excretion of uric acid by human subjects is partly regulated by endocrines. The administration of 11-hydroxysteroids of the adrenal cortex, or of ACTH (see Chapter 23), causes an increase. It is not known whether this is due to an increased biosynthesis or to an increased elimination.

Coffee, tea, and cocoa contain methylated purines and also some of the amino- and oxypurines. In nature methyl purines occur as follows: caffeine in coffee, tea, kola nuts, cacao, and Maté (Paraguay tea); theobromine in cocoa or chocolate bean; theophylline in minute amounts in coffee, tea, and chocolate. The various cola drinks which are so popular contain caffeine. Caffeine and theophylline are not converted to uric acid, according to Myers and Hanzal, and this is probably true also of theobromine. Probably 1-methyl- and 1,3-dimethyluric acid are the final products of the metabolism of caffeine and theophylline.

The fate of pyrimidines in metabolism is very obscure. Small amounts of uracil and thymine are easily disposed of by the organism, but larger amounts are not. Cytosine seems to be particularly resistant to breakdown by the body. But apparently the pyrimidines are not set free in digestion or in metabolism. Deuel was unable to find even a trace of pyrimidine in 150 liters of human urine. D. W. Wilson believes that radical changes probably take place in both the pyrimidine and purine groups of a large part of the nucleotides and nucleosides before they are split.

### Synthesis of Purines and Pyrimidines

The purines of the body can be formed in the absence of preformed purines of the diet. An excellent example is the bird egg, which begins as a single cell with one nucleus, containing a minute amount of nucleoprotein, and, during its development, progressively increases in the number of cells and amount of nucleoprotein. The salmon, during its long travels from the sea up the rivers to spawn, forms large amounts of nuclein-rich sperm and roe which



must be derived from its own tissues because it eats nothing during this period. Experimentally, synthesis of purines has been demonstrated in man and in the Dalmatian hound, which differs from other breeds of dogs in having uric acid as its purine end product.

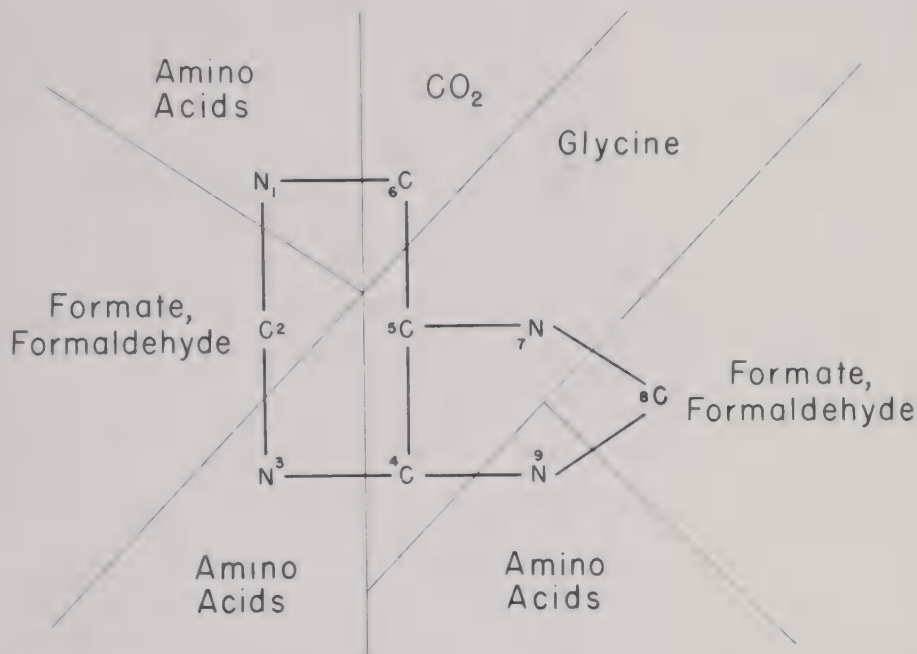
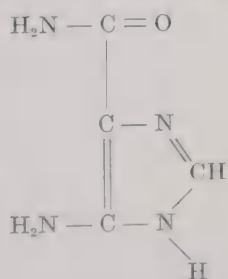


Fig. 48.—Diagram of the purine skeleton indicating, in general, the sources of the different atoms in the biosynthesis of purines.

Until recently biochemists sought for some preformed precursor of the purines in the diet. That is, they felt that some fairly large molecules, having part of the configuration of these larger molecules, should logically form their building stones. Thus it was believed—and there was some experimental support for the belief—that arginine and histidine were possible precursors. A comparison of their structural formulas with the purine nucleus will show the basis for this conception. Modern experiments using compounds labeled with isotopic nitrogen and carbon have shown that neither arginine nor histidine can play such a role. Smaller molecules, as small even as  $\text{CO}_2$ , are utilized to build up the large purine molecules. A probable intermediate in these synthetic reactions is 4-amino, 5-imidazolecarboxamide (Schulman and Buchanan):

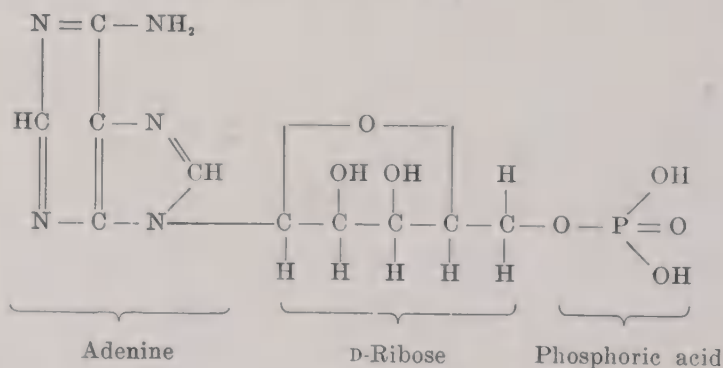


Recently Barnes and Schoenheimer have fed ammonium salts marked with  $^{15}\text{N}$  to pigeons and to rats. Pigeons were first used because birds (and reptiles) excrete a large part of their waste nitrogen as uric acid rather than as urea.

The isotopic nitrogen was found in the uric acid of the excreta of the birds, as well as in the purines and pyrimidines isolated from the nucleic acids of their nucleoproteins. In rats the same thing occurred, except that purine and pyrimidine synthesized was not as great because of the fact that a large fraction of the isotopic nitrogen went to form urea. In the rat, ammonia nitrogen, when fed, is rapidly converted into amino acids of the tissues and apparently thus enters into numerous interreactions in the body. When  $N^{15}$ -labeled glycine is fed to man, the urinary uric acid contains  $N^{15}$  in the 7 position (Shemin and Rittenberg, 1947). The other nitrogen atoms are derived from aspartic acid, glutamic acid, or glutamine more readily than from ammonia or ammonium compounds. Glycine has been shown to contribute its carboxyl carbon to form C4, and its alpha carbon to form C5, and thus glycine provides atoms 4, 5, and 7. Formic acid and formaldehyde are very important precursors of C2 and C8, the two ureide carbons. Carbon dioxide yields its carbon to form C6. Thus it appears that the precursors of purines are glycine and other amino acids,  $CO_2$ , formic acid, and formaldehyde, as well as a number of other intermediaries which yield these compounds in metabolism. (Buchanan.) (See Fig. 48.)

The pyrimidines, which appear so similar in structure to the purines, have been shown by Heinrich and Wilson not to have the same precursors as the purines, except in the case of  $CO_2$ . Here, however, the C becomes the ureide carbon C2.

**Nucleotides.**—Some free nucleotides occur in nature. Liebig in 1847 isolated inosinic acid from beef extract. Since that time several others have been discovered, among them guanylic acid from pancreas, spleen, liver, and yeast and adenylic acid from brain and muscle. Muscle adenylic acid is:



Adenylic acid may be converted to inosinic acid by a deaminase found in muscle, thus changing the adenine portion of the molecule to hypoxanthine. Substitution of the latter purine, therefore, for adenine in the above formula gives the formula for inosinic acid. In yeast guanylic acid the purine is guanine, and the phosphoric acid is attached to carbon 3 instead of carbon 5 of the pentose.

Adenylic acid is a biological substance of prime importance. It possesses the property of adding and subsequently releasing two additional phosphoric acid molecules with their quota of 9,000 to 12,000 calories of energy each. This enables it to participate in energy transformations as previously described

(see page 354) and phosphorylation reactions (see page 355). New roles for this unique substance are continually being discovered. Recently Neuberg and Mandl have observed a remarkable capability of adenosine triphosphate (ATP) to dissolve insoluble metal salts and to prevent their normal precipitation. These include the carbonates and phosphates of magnesium and calcium, as well as the same salts of heavy metals, including  $\text{Fe}^{++}$  and  $\text{Fe}^{+++}$ . A number of other salts are likewise affected; also certain sulfates, sulfides, and stearates. Some alkali salts of meta- and pyrophosphoric acid have similar solubilizing action upon a wide variety of inorganic salts. In these inorganic condensed phosphates, which occur in many cells, as does ATP, energy is stored and the mechanism is probably similar in all cases.

Coenzymes I and II, or DPN and TPN, respectively, are also nucleotides but of a more complex nature. Their structure is shown on page 347, where their function as hydrogen carriers is discussed.

### Clinical Uses of Amino Acids

Myasthenia gravis is a pathological condition, marked by excessive tiring of the voluntary muscles and rapid decrease in their contractility. The feeding of glycine is said to be of therapeutic value in this condition sometimes. Glycine is one of the precursors of creatine, which enters into the physiological activity of muscle.

The parenteral feeding of amino acids is a recent development with great potentialities. The most obvious reason for parenteral feeding of any kind is the inability of a patient to take food or fluid by mouth because of vomiting or obstruction of some part of the gastrointestinal tract. Other conditions in which it is indicated are severe diarrhea, intestinal fistula, peritonitis, and when surgery requires complete rest of the tract. For many years saline solutions and glucose solutions have been administered intravenously or hypodermically to furnish fluid, minerals, and carbohydrates. The glucose so given would theoretically spare the destruction of body protein, but practically it is not of much importance from this standpoint. Moreover, there is usually a sufficient amount of glycogen and fat to furnish energy for a long time. Nitrogen, however, is constantly eliminated as urea, creatinine, ammonia, uric acid, etc., and must be derived from the protein of the patient's tissues. In the ten days following a serious operation as much as 1,100 Gm. of protein may be lost, corresponding to about twelve pounds of muscle tissue or its equivalent. This comes not only from muscle, but also from all types of tissue. Among these, plasma protein is of primary importance. Hypoproteinemia has been observed in many such cases with its attendant production of edema. Loss of liver protein would tend to provoke or aggravate hepatic insufficiency. In extensive burns there is an enormous loss of nitrogen. Sometimes as much as 250 Gm. of protein a day is lost because of tissue destruction and gross abnormalities in nitrogen metabolism. As much as 80 per cent of the nitrogen of the urine in cases of burns may be in the "residual" nitrogen; that is, nitrogenous constituents, the character of which is unknown. The negative nitrogen balance in such cases is very great, despite high protein and high caloric diets. The



parenteral injection of plasma proteins or of amino acids undoubtedly is indicated for those patients who cannot eat and perhaps, also, after burns or trauma, in which the negative balances are so great. Plasma proteins of the same species appear to be well utilized, but there are two objections to the use of blood plasma proteins. They are expensive, or difficult to obtain under normal conditions, and they are nutritionally not as good as many other proteins. The intravenous use of amino acids is probably a more promising procedure. Casein digests, which contain all the essential amino acids, and which are nontoxic to man, can now be obtained. Patients have been brought to nitrogen equilibrium and have been maintained in that state up to twenty-three days (Elman). In some cases glucose and even emulsified fat have been added to the injection fluid. Plasma protein regeneration as well as clinical improvement have been observed. The inclusion of as much as 75 to 150 Gm. of fat in the intravenous infusion mixture has been accomplished without serious reaction and affords a considerable number of calories (Shafiroff.)

Based on experimental studies upon animals, there have been a number of reports showing the favorable effect of feeding (or in some cases injecting) certain amino acids to patients suffering from hepatic diseases of one sort or another. In such cases the feeding of a diet high in protein to supply enough of the particular amino acids needed is usually nearly impossible. Thus there are several favorable clinical experiences with cystine and choline in the treatment of cirrhosis of the liver. (Beams.) This follows the observations of György and Goldblatt that choline is more effective when administered along with cystine.

Glutamic acid hydrochloride seems to have a special relationship to nervous function. It appears to improve the condition of patients suffering from the mild form of epilepsy. (Price.) The use of tryptophan as a niacin precursor in pellagra has already been mentioned (page 291).

## ESSENTIAL AMINO ACIDS

In Chapter 5 a division between dispensable and indispensable amino acids was made. It is now advisable to clarify some phases of this subject and, perhaps, modify some rather arbitrary statements. Among the latter was the statement that the indispensable amino acids cannot be synthesized by the body. However, it has just been seen (page 390) that young animals will grow on a methionine-free diet if homocystine and either choline or betaine are present. The reason for that is plain, but it indicates that special conditions may arise whereby the body might be able to synthesize an indispensable amino acid if all of the special "ingredients" were at hand and conditions were favorable.

Block and Bolling suggest a classification of the amino acids into dispensable, semidispendable, and indispensable (see Table XXXIII). Arginine is called semidispendable because it is synthesized by man but not at an optimal rate. Glycine is not essential for the rat or the human being under usual conditions but is necessary for optimal growth of fowls. Another group of

amino acids is semidispendable from another standpoint. These are dispensable only if a closely related amino acid is provided in the diet in quantities sufficient to cover the needs of both. Cystine, for example, has no effect upon growth if methionine, from which it can be synthesized, is abundantly supplied, but cystine will stimulate growth if methionine is present in the diet in insufficient amounts. Tyrosine bears a similar relationship to phenylalanine. It has also been seen that histidine is not essential in the diet of man, probably because it is synthesized by intestinal bacteria, and methionine is not necessary in the diet if homocystine and choline are supplied. For the present, methionine should be considered an essential amino acid for man.

TABLE XXXIII  
TENTATIVE CLASSIFICATION OF AMINO ACIDS\*

DISPENSABLE	SEMIDISPENSABLE		INDISPENSABLE (FOR MAN)
	GROUP A	GROUP B	
Glutamic acid	Arginine	Cystine	Lysine
Aspartic acid	Glycine	Tyrosine	Tryptophan
Alanine	Histidine		Phenylalanine
Serine			Methionine
Proline			Threonine
Hydroxyproline			Leucine
			Isoleucine
			Valine

\*Adapted from Block, R. J., and Bolling, D.: J. Am. Dietet. A. 20: 69, 1944.

It should be noted that a mixture of amino acids, deficient in one or more of the essential ones, is of little nutritive value as regards its ability to build or replace body proteins. Such a mixture is deaminized to a considerable extent and used for the production of energy but does not contribute to a positive nitrogen balance. Even a subsequent administration of the missing essential amino acids is of no avail, because amino acids are not stored in the tissues for any considerable length of time. The indispensable amino acids must be given as a complete mixture in approximately the proper proportions. This applies to either the oral or parenteral administration of amino acids or protein hydrolysates and also to the feeding of incomplete proteins (like gelatin), when unaccompanied by other proteins to supplement them.

The dispensable amino acids must not be considered of no value. It would be better to designate them as "synthesizable," or by some similar adjective. For the synthesis of the nonnitrogenous portion, both the carboxyl and the methyl carbons of acetate, and, to a less extent, the carbon of bicarbonate are needed. This was determined by using  $C^{14}$  labeled salts. (Greenberg and Winick.) The  $NH_2$  is probably obtained from deamination reactions in the liver. These amino acids go to make up body protein, as do the indispensable ones, but they are more interchangeable, more versatile, as well as more easily procurable. Glycine, one of the semidispendable, is used to make creatine and to detoxicate benzoic acid. Citrulline forms a part of the arginine-urea cycle, and other examples of their specific usefulness could be mentioned. But if the diet lacks any of these, the body can synthesize them from others.

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## Chapter 16

### CARBOHYDRATE METABOLISM

The foundations of the study of carbohydrate metabolism were laid by Claude Bernard (1813-1878), the great French scientist, who perhaps may be called the first biochemist. Bernard's experiments began with an attempt to produce in animals a condition analogous to human diabetes mellitus. In this condition the outstanding symptom is the passage of glucose into the urine. At that time nothing whatever was known regarding its cause. In the course of his studies Bernard found that if the floor of the fourth ventricle of the medulla oblongata of rabbits was punctured, a temporary diabetes resulted. This "piqûre," as it was called, could be produced only in animals which were in good nutritive condition—not in starving animals. His next step was to make a survey, so to speak, of the concentrations of the glucose of the blood. This, under normal conditions, is about 0.10 per cent (100 mg. per 100 ml.). But he found that there were slight differences between venous and arterial blood; for example, blood taken from the carotid artery might have a concentration of about 120 mg. per cent, while that from the jugular vein nearby had 80 mg. per cent. It appeared that the blood, in going from the arteries into the tissue capillaries and back to the veins, had left some of the glucose behind for the use of the tissues. By passing sounds through the heart down the vena cava and withdrawing samples of blood at different levels, Bernard ascertained that the blood taken from a level opposite the kidneys contained about the same percentage of sugar as the venous blood in general. However, blood taken from a point near the opening of the hepatic vein contained more glucose (140 mg. per cent) than the arterial blood. Since the portal blood contained less sugar than the blood from the hepatic vein, the inference was that the sugar had been added while the blood was passing through the liver. Some substance present in the liver was being converted into glucose. The next step was to find this source of glucose in the liver. A well-fed rabbit was killed instantly and the liver removed as rapidly as possible, plunged into boiling water, and cut up in it while the water was boiling. An opalescent solution resulted. This had very little reducing sugar in it but it did contain some compound which yielded a reducing substance upon hydrolysis. This was the material which caused the opalescence. Bernard called it the "glycogenous matter." It is what we now call glycogen. If the procedure described was not carried out with dispatch, little or no glycogen could be found, but reducing sugars were present in abundance. This indicated the presence of a glycogen  $\rightarrow$  glucose mechanism which could be regulated in the living organ and which boiling inhibited in the removed tissue. This, today, we recognize as an enzyme reaction. Bernard formulated the glycogenic theory, that glycogen is the form in which glucose is stored in the liver and that it can be changed to glucose which is carried to the various organs and tissues for conversion to heat and en-

gly or for reconversion to the glycogen of the tissues. Since Bernard's time, many additional facts have been gathered and many theories have been evolved to explain the details of the various steps. But the broad features of Claude Bernard's glycogenic theory remain just as true today as when they were first developed in the middle of the last century.

## ABSORPTION

Digestible carbohydrates are brought to the monosaccharide stage in the intestinal canal before they are absorbed. In fact, no higher carbohydrate can be absorbed, and if administered parenterally it is eliminated as a foreign body. Even the disaccharides cannot be utilized when injected intravenously or administered otherwise than by mouth. Probably all the monosaccharides are absorbed to some extent by simple diffusion. However, they are not all absorbed at the same rate—galactose is taken up most rapidly, glucose next, fructose still more slowly, then mannose, and the pentoses come last. It is evident that if absorption were merely a process of diffusion the pentoses with smaller molecules could be absorbed more rapidly than the hexoses, and there would be no difference between the individual hexoses. The reason for the difference seems to be in the fact that the utilizable sugars are absorbed chiefly by a special mechanism. They are phosphorylated; that is, they are combined with phosphoric acid during absorption. These are principally glucose, fructose, and galactose. The pentoses are not so combined and wander into the body by diffusion. Although absorbed very slowly, mannose appears to be utilized exceedingly well by the rabbit (Bailey and Roe). A seven-carbon sugar, D-mannoheptulose, occurring in the avocado, is absorbed and apparently utilized to some extent by normal persons. (Blatherwick.) This is a keto sugar. Interestingly, Roe and Hudson have found that the corresponding aldose, D-mannoheptose, is not utilized at all by rabbits, although the ketose is.

Glucose absorption by the intestinal mucosa is brought about by a phosphorylase in the presence of adenosine triphosphate (ATP). ATP will be discussed in detail later, but, for the present, it will suffice to say that it is a donor of phosphoric acid and becomes adenosine diphosphate (ADP). This phosphorylation is not under the control of insulin. Fructose and galactose are similarly phosphorylated. The reactions for glucose and fructose are schematically shown below:

Intestinal lumen	Mucosal cell		Circulating Blood
Glucose (From food)	+ ATP Phosphorylase	Glucose-6-phosphate + ADP	Glucose + Phosphate
Fructose (From food)	+ ATP Phosphorylase	Fructose-6-phosphate + ADP	
	Isomerase	Glucose-6-phosphate	Glucose + Phosphate

After absorption by the intestinal mucosa the monosaccharides are carried to the liver in the portal blood. In the liver the utilizable hexoses are converted, in part, to glycogen. This is the form in which the carbohydrates are stored in the animal. There is some evidence that the glycogens formed from glucose, fructose, and galactose differ somewhat (Deuel), but we are fairly certain that glycogen always breaks down to glucose, no matter what its origin. During this period of absorption it is impossible for the liver to remove all the sugar from the blood passing through it. Some gets into the systemic blood and there is a rise in the concentration of blood sugar. In the muscles a second supply of glycogen is stored which has a special local function. There are usually about 200 Gm. of glycogen in the body of a normal adult, about half in the liver and half in the muscles, with very small amounts in other soft tissues and traces in the blood.

The general pathway of carbohydrate is given in Fig. 49. Relationships to lipid and protein metabolism are omitted, but they are discussed elsewhere. The liver glycogen is changed back to glucose which is carried to the muscles (and other tissues). Here it forms muscle glycogen, a source of energy for muscle contraction. When glycogen is broken down, lactic acid is formed, part of which is oxidized and part sent back to the liver to be changed to glycogen again.

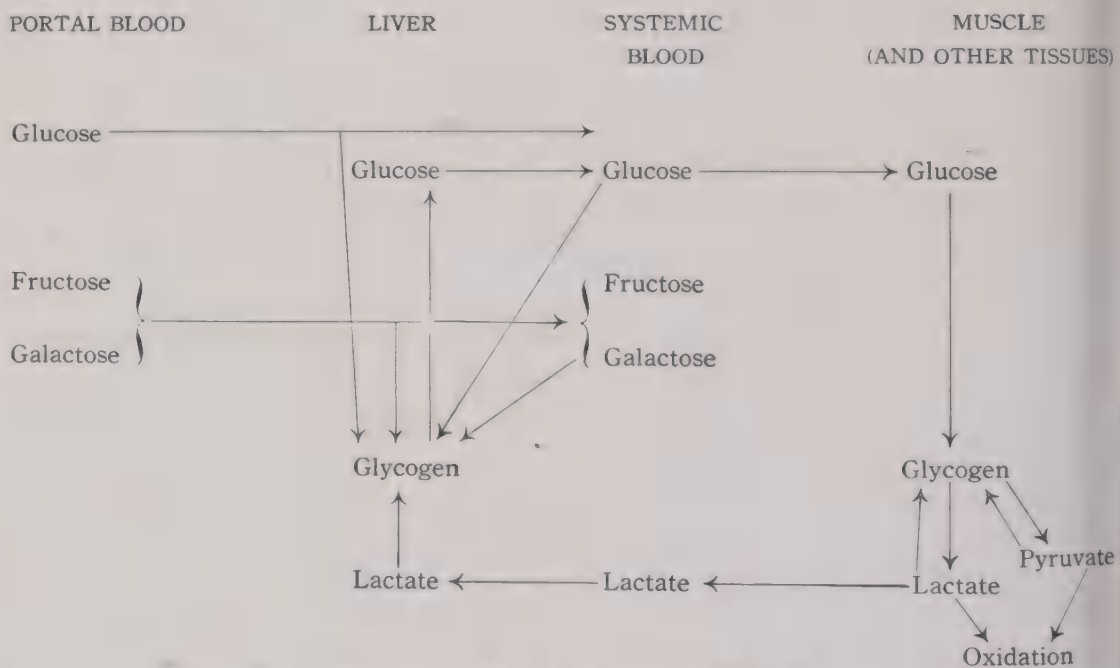


Fig. 49.—General path of carbohydrates in the body.

It will be noted that the reaction  $\text{glucose} \rightleftharpoons \text{glycogen}$  is represented as being reversible in the liver, but in the muscle it is irreversible,  $\text{glucose} \rightarrow \text{glycogen}$ . This refers to the fact that muscle glycogen is not a source of blood sugar.

### The Blood Sugar

It is generally accepted now that the sugar of the blood is  $\alpha\beta$ -D-glucose, in addition to minor quantities of sugar phosphates, but there may be traces



the other hexoses, depending chiefly upon the amounts in the diet. The concentration is fairly constant. Samples of blood taken before breakfast usually contain from 0.07 to 0.10 per cent glucose, or, as it is generally stated, from 70 to 100 mg. per 100 ml. of blood. During the day it will range from 0.07 to 0.16 per cent, i.e., from 70 to 160 mg., although usually it seldom rises above 130 mg. After a meal there is a sharp rise followed by a gradual fall, so that in one or two hours the concentration is back to the 70 to 100 mg. per cent level. These figures refer to venous blood. Capillary blood usually is about 10 mg. higher, because it more nearly resembles arterial blood. During sleep the blood sugar reaches a low level which is maintained usually until breakfast the next day. In Fig. 50 it is shown approximately how the blood sugar varies with meals and with the time of day.

**The Constancy of Blood Sugar Level.**—There are a number of factors influencing the level of blood sugar which are so delicately balanced that it ordinarily stays within the limits from 70 to 130 mg. per cent. These include the following:

- The glycogen  $\rightleftharpoons$  glucose reaction in the liver
- The formation of glycogen in muscle and its utilization
- The utilization of carbohydrate by other tissues
- The conversion of carbohydrate to fat
- The excretion of glucose

Indirectly a number of other factors operate, including gluconeogenesis, interconversion of fat and carbohydrate, and interplay of hormones (insulin, epinephrine, adrenal cortical hormones, growth hormone, etc.). Blood sugar values above the normal range are termed hyperglycemias, and those below it are spoken of as hypoglycemias. The hyperglycemia following an increased intake of carbohydrate in the diet is called an "alimentary hyperglycemia." Bodansky has shown that galactose most easily produces this condition, with glucose next and fructose last. This is in harmony with their rates of absorption.

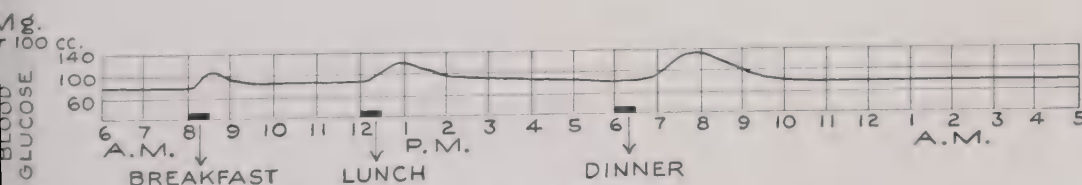


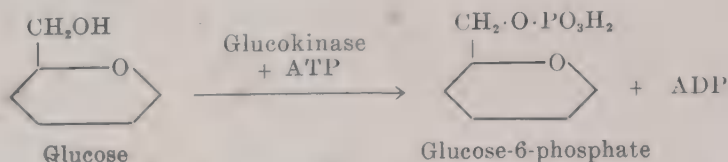
Fig. 50.—Typical blood sugar variations in a normal young adult throughout the day.

## GLYCOGEN FORMATION IN THE LIVER

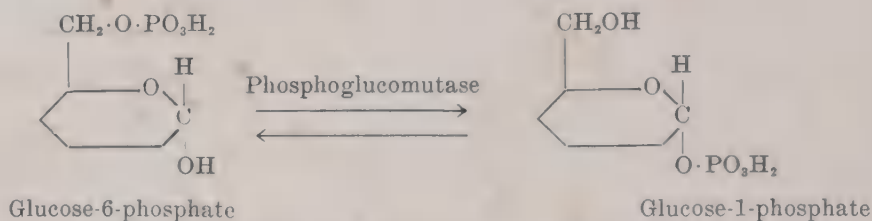
### Phosphorylation and Phosphorolysis

The addition of phosphoric acid to glucose is essential to its utilization. This is called "phosphorylation," a term coined by Neuberg in 1910. As we shall see, the addition and subtraction of phosphoric acid is necessary in most of the steps of carbohydrate metabolism, and accompanying them and bound up in them are the important transfers of energy.

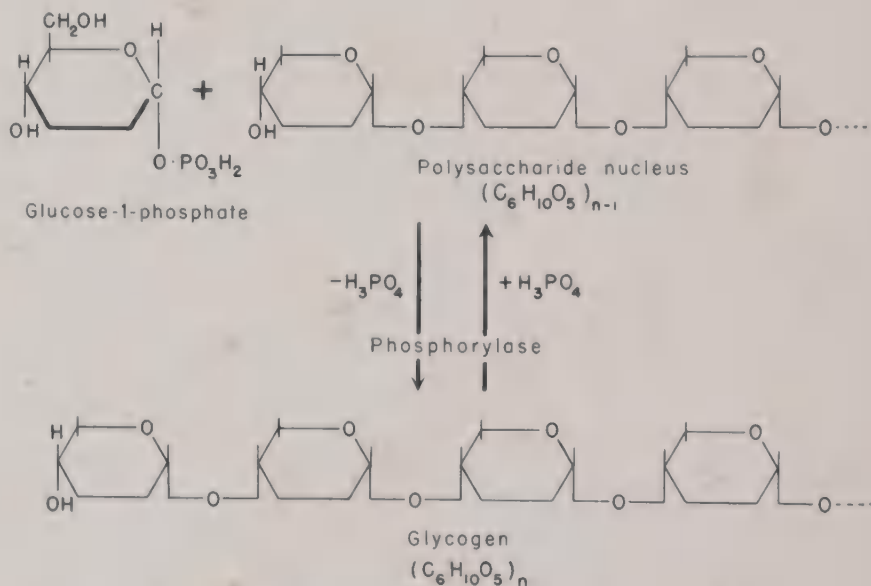
For the phosphorylation of glucose, a hexokinase (in this case glucokinase), and adenosine triphosphate (ATP) are required. Phosphoric acid is attached at carbon 6, thus:



The next step is a transfer of the phosphate group to carbon 1, which is brought about by phosphoglucomutase.



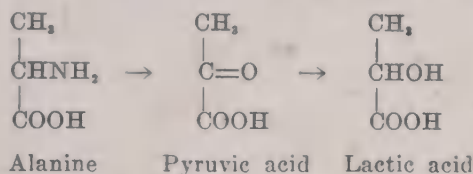
Glucose is then ready for transformation into glycogen—a process known as “glycogenesis.” The monosaccharides present in the portal blood are absorbed by the liver cells and converted into glycogen; or rather, polysaccharide chains, already present, are lengthened by the addition of glucose-1-phosphate, since the reaction does not occur in the absence of a small amount of polysaccharide “primer.” The underlying mechanism is a dephosphorylation, an action of another phosphorylase; thus:



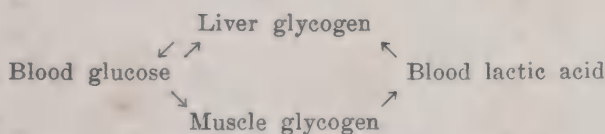
The reaction in the reverse direction also is called a *phosphorolysis*, being analogous to the *hydrolysis* of polysaccharides; the former involves phosphoric acid; the latter, water.

Glycogenesis also occurs when the glycemia, that is, the level of blood sugar of the general circulation, rises above normal.

The hexoses are not the only glycogen formers. We have seen that proteins yield glucose approximately to the extent of 58 per cent of their weight. This is because of the nonnitrogenous fraction left after deamination of certain of the amino acids. A good example is alanine. This is converted to pyruvic acid by oxidative deamination. Pyruvic acid, in turn, can be transformed to lactic acid. Both pyruvic acid and lactic acid are known glycogen formers.



The glycerol fraction of fats is also convertible to glucose and therefore to glycogen. The fatty acid portion is probably not changed directly to glucose, but the non-nitrogenous fragments of the metabolism of all foodstuffs enter the final common pathways of metabolism. (See page 463.) The formation of glucose from noncarbohydrate sources is given the term "gluconeogenesis." Lactic acid, which is a product of carbohydrate catabolism, especially in muscle, has been found to be transported in the blood stream back to the liver for reconversion to glycogen (Himwich; Cori).



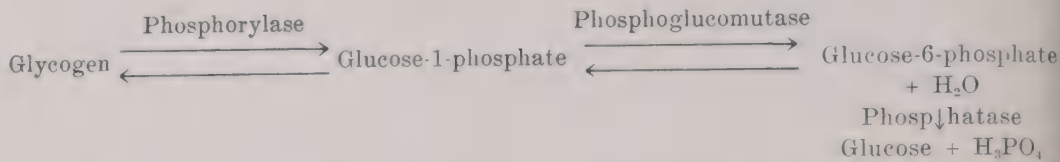
Experiments of Hastings and his group have brought forth some interesting facts concerning the conversion of lactic acid to glycogen. Lactate, containing radioactive carbon in the carboxyl group, was fed to rats and the radioactivity of the liver glycogen and of the expired  $\text{CO}_2$  was studied. Results indicated that only a small amount of the lactate is converted into glycogen as a three-carbon chain. Some of the carboxyl is split off and excreted as  $\text{CO}_2$  into the expired air. Thus glycogen may be formed from two-carbon chains. It is even possible that  $\text{CO}_2$  may enter into the formation of glycogen. When nonradioactive sodium lactate was fed and radioactive sodium bicarbonate was injected intraperitoneally, a small amount of the radioactive carbon appeared in the liver glycogen. The work of Evans (page 542) on the utilization of  $\text{CO}_2$  points in the same direction.

## GLYCOGENOLYSIS IN THE LIVER

Glycogen is transformed into glucose-1-phosphate in the presence of inorganic phosphate by a phosphorylase. This was formerly called glycogenase but the new terminology describes its action more accurately. This enzyme is widely distributed in plants, microorganisms, and in animals. In animals it is found in muscle, heart, liver, and brain. The reaction occurs very rapidly and is the classical reaction described by Claude Bernard. Adenylic acid is a cofactor for it. The reaction is that shown on page 416 but continues to glu-



cose-6-phosphate. The phosphorolysis of glycogen is accelerated when the body requires more glucose. Such is the case during muscular activity or exposure to cold, both of which tend to lower the blood sugar level. The glucose-6-phosphate, hydrolyzed by a phosphatase to glucose and phosphoric acid, raises the blood sugar to a normal level again.



Glycogenolysis, as this disintegration of glycogen is called, is under the control of adrenaline through the sympathetic nervous system. Stimulation of the sympathetic nerves causes the increased secretion of adrenaline, the hormone

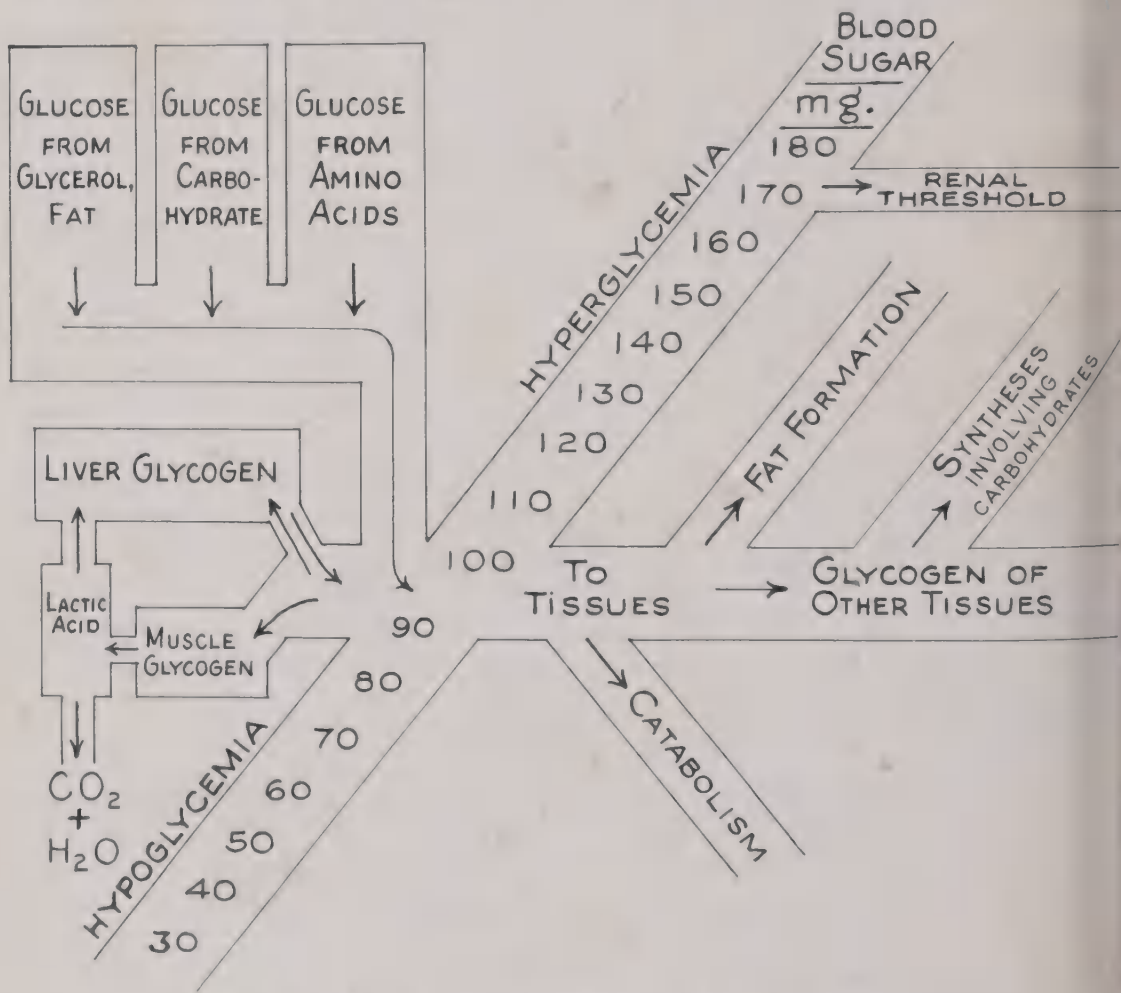


Fig. 51.—Diagram of carbohydrate metabolism.

of the adrenal medulla. This augments glycogenolysis by some mechanism as yet unknown. Violent emotional reactions, such as fear and rage, lead to the formation of adrenaline which, in turn, increases the blood sugar. This, according to Cannon, is an emergency reaction to furnish extra fuel to enable

the animal or person to escape from danger or to fight, as the case may be. Insulin secretion has an opposite effect, tending to cause glycogenesis. Thus these two hormones seem to balance each other.

The absence of the specific phosphatase which converts glucose-6-phosphate to glucose and phosphoric acid in the liver has been demonstrated in cases of glycogen storage disease. This is a rare congenital disorder of metabolism in which there is enlargement of the liver, due to the storage of glycogen. There are usually hypoglycemia and acidosis. It is a rather rare condition, but even more infrequent is the occurrence of a cardiac type; that is, glycogen is stored in the heart. This type is rapidly fatal. Other varieties of glycogen storage disease appear to be due to the deposition of abnormal forms of glycogen. (Cori.)

### GLYCOGENESIS IN MUSCLE

Glycogen formation in muscle differs from that in liver in that no sugar other than glucose can be used for this purpose. However, glycogen is formed in the muscles when fructose is injected into hepatectomized animals. This would seem to point to the direct utilization of this sugar as well as glucose. This is not the case because if the hepatectomized animal is also deprived of intestines, fructose is not a glycogen former (Bollman and Mann). The explanation is that the intestines (and also the liver) are capable of transforming fructose to glucose, which then enters into muscle glycogenesis. Colowick and Berthland have succeeded in converting glucose to glycogen in vitro with the aid of purified hexokinase, phosphoglucomutase, and the special phosphorylase. Therefore, glycogen synthesis in muscle is accomplished by three or more enzyme reactions. In frog muscle, glycogen may be *rebuilt* from some of the lactic acid which is produced when glycogen is broken down. Formerly this was thought to be the case in mammals also, but it has been shown that this occurs to only a slight extent. The hormones, adrenaline and insulin, have the same type of control over muscle glycogenesis as they do over liver glycogenesis, adrenaline diminishing and insulin increasing it. When the muscles contract they use up some of their stored-up glycogen. To replenish this, blood sugar is drawn upon, and in order to keep the blood sugar at a normal level, the liver pours more glucose into the blood stream.

### GLYCOGENOLYSIS IN MUSCLE

Glycogenolysis in muscle is essentially the same as glycogenolysis in liver. It is a phosphorylation reaction with the production of glucose-1-phosphate and glucose-6-phosphate. However, there is one great difference. The phosphatase, which in liver converts glucose-6-phosphate into glucose and phosphoric acid, is absent in muscle. Therefore glucose is not set free in muscle and it is not sent to the blood to augment the blood sugar. The glucose-6-phosphate is broken down further through a number of intermediate steps to lactic acid, and eventually the latter is further decomposed. Evidence that muscle glycogen is not a source of blood sugar was furnished by the experiment of Bollman, Mann, and Rath. When the liver was removed from a dog, the blood sugar concentration

fell steadily to a very low level. The muscle, however, was found to contain considerable amounts of glycogen. Evidently it was not available for conversion into blood sugar.

**Excretion of Glucose.**—Ordinarily the amount of glucose in urine is negligible. The range has been shown to be from 0.01 to 0.10 per cent (Neuwirth) and certainly is not in sufficient concentration to give a positive reaction with Benedict's qualitative test. Normally the blood sugar passes through the glomeruli of the kidneys and, in aqueous solution, flows into the tubules. Here it is reabsorbed. A phosphorylation, that is, a combination with phosphate, is necessary for absorption from the tubules just as it is for absorption from the intestinal canal. If reabsorption cannot keep pace with glomerular secretion, glucose will, of course, find its way into the urine. This is called "glycosuria" or, more exactly, "glucosuria." The chief reason for its occurrence is an increased secretion of sugar as a result of a high blood sugar. Phosphorylation and reabsorption cannot keep pace with the secretion and the excess sugar flows into the urine. There is usually a fairly definite level of blood sugar for a given individual, above which sugar is excreted. This "renal threshold" is usually found to be between 140 and 180 mg. per cent glucose, averaging 160 mg. per cent. It frequently is higher with older people or if the kidneys are damaged. For this reason an abnormally high blood sugar is sometimes found when the urine is quite free from reducing substances. This indicates a disturbed carbohydrate metabolism just as definitely as does glucosuria. If the renal threshold is found to be normal, an examination of the urine obviates the necessity of frequent blood analyses.

**Conversion of Carbohydrate to Fat.**—Another physiological mechanism which tends to keep the blood sugar at a constant level is the transformation of excess glucose into fat. This is such a well-known phenomenon that it scarcely needs emphasis. The fattening of hogs and the production of cream are instances known to every farmer. The European custom of stuffing geese with bread or other starchy foods to produce the fatty goose liver is another example. In these cases glycogenesis in the liver and muscles occurs first. When these tissues are not capable of storing more glycogen, fat formation begins. The mechanism of this action is not known but undoubtedly hormonal influences play a part, the conversion being effected by enzymes. We may assume that the formation of fat from an excess of carbohydrate is a normal process ordinarily. That is, it is a provision of nature to enable the individual to store large excesses of carbohydrate in the form of fat, the high calorie food, which can be drawn upon when the more readily convertible storage food, glycogen, has been depleted. This would imply that simple cases of obesity result from a normal tendency to store food. The fact that some individuals have great difficulty in putting on weight is probably just as abnormal as the fact that others become obese.

### UTILIZATION OF GLUCOSE

By "utilization" of glucose reference is made only to those transformations whereby useful energy and heat are produced. It must be remembered, however, that the sugar molecule may be required for building glycoproteins.



lycolipids, glucosamine, etc., and fractions of the molecule may be used wherever a short carbon chain could fit in. Similarly, we do not include in utilization the storage of carbohydrate as glycogen, or fat. The word "oxidation" is avoided because many of the transformations are not oxidations in any of the meanings of that term. Some reactions must necessarily be oxidations because we start with carbohydrate and end with carbon dioxide and water. The broad vital reaction is



However, neither glucose nor glycogen reacts directly with oxygen. Intermediate products are first formed without oxidation, and oxidation is a later stage in the decomposition.

The breakdown of glycogen or of glucose takes place in all tissues. The reactions have been studied more actively in striated and cardiac muscle and in nervous tissue than in other tissues. It is evident, however, that they must occur in all parts of the body. Possible glycogenolysis and the subsequent disintegration of glucose, called "glycolysis," are qualitatively the same in many of them. The amount of energy transformation is much less in the glands, bones, and connective tissues than in muscles, and the activity of the spleen is less than that of the kidney. But carbohydrate metabolism is occurring in all of them all the time, and some of the reactions to be discussed will probably be found to be applicable to all of them.

Glycolysis in the tissues resembles fermentation (see page 61) to a certain extent. Trioses are formed and, eventually, pyruvic acid. In fermentation this is converted, in part, to ethyl alcohol and carbon dioxide. There is a possibility that traces of ethyl alcohol are formed in mammalian tissues, but probably the main pathway from pyruvic acid is through lactic acid and eventually to  $\text{CO}_2$  and water. (See also Chapters 14 and 20.)

### Carbohydrate Metabolism in Striated Muscle Contraction

The conception of muscle metabolism held until recent years was that the energy was derived from the combustion of glycogen, resulting in the production of carbon dioxide and water. Although glycogen is eventually converted to these products and oxygen is utilized in the transformations, it is by no means a direct oxidation. There is, in fact, at the moment of contraction, no combustion of fuel by the muscle; that is, there is no absorption of oxygen or production of  $\text{O}_2$ . Contraction of an isolated muscle can occur in the complete absence of oxygen. This may continue for an appreciable length of time, during which the glycogen is changed to lactic acid (lactate), which accumulates. If this is continued too long, *rigor mortis* sets in. However, if the contractions occur in the presence of sufficient oxygen, part of the lactic acid is reconverted to glycogen and part is oxidized. Normal muscle contraction, therefore, may be divided into two phases:

1. Contractile, or anaerobic
2. Recovery, or aerobic

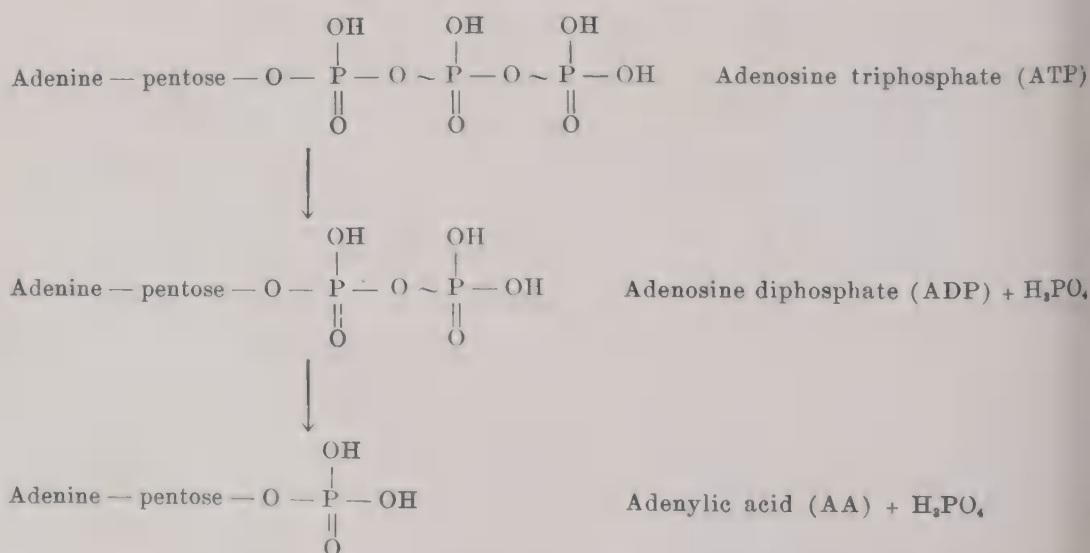
Although the contractile phase is called anaerobic and oxygen is not needed for it, it must be emphasized that it can and ordinarily does occur in the presence

of oxygen. For the recovery phase oxygen is necessary and carbon dioxide is formed. Consequently the energy for contraction cannot originate in an oxidation but must come from the sudden breakdown of some labile molecule.

For some time it was thought that the energy for muscle contraction came from the breakdown of creatine phosphate. At present, however, adenosine diphosphate and triphosphate are considered the agents which actually release energy, although other compounds may be involved in the intricate process.

**Creatine Phosphate.**—In 1927 Eggleton and Eggleton, in England, discovered in muscle an organic phosphate which was easily hydrolyzed. They called this “phosphagen.” The same year Fiske and Subbarow, in this country, made the same discovery and were able to identify the substance as creatine phosphate. It is such an unstable compound that the muscle must be frozen before removal for analysis, and even under these conditions there is evidence that some of it has been hydrolyzed. Meyerhof showed that the decomposition of creatine phosphate to creatine and phosphoric acid, whether accomplished by an enzyme or by an acid, was accompanied by the release of heat. Muscle extract contains this enzyme, but if it is dialyzed it becomes inactive. In the dialysate, magnesium ions and adenosine triphosphate can be found, which are needed for this enzymic reaction. Adenosine phosphates are required for all such transformations in which the transfer of phosphate is accompanied by a release of energy. Thus we see why creatine phosphate was at first thought to be the source of muscle energy. It is now considered to be an active phosphate carrier, capable of transferring its energy to other similar carriers or to adenylic acid or adenosine diphosphate (see also Chapter 14).

**Adenosine Triphosphate.**—Adenosine triphosphate is a nucleotide, a molecule of which contains adenine, pentose, and three molecules of phosphoric acid. It can release two of these molecules in succession. Thus:

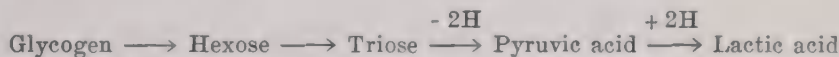


Because of the presence of two “energy-rich phosphorus” bonds (see page 354), each of these hydrolyses is accompanied by the transfer of a large amount of

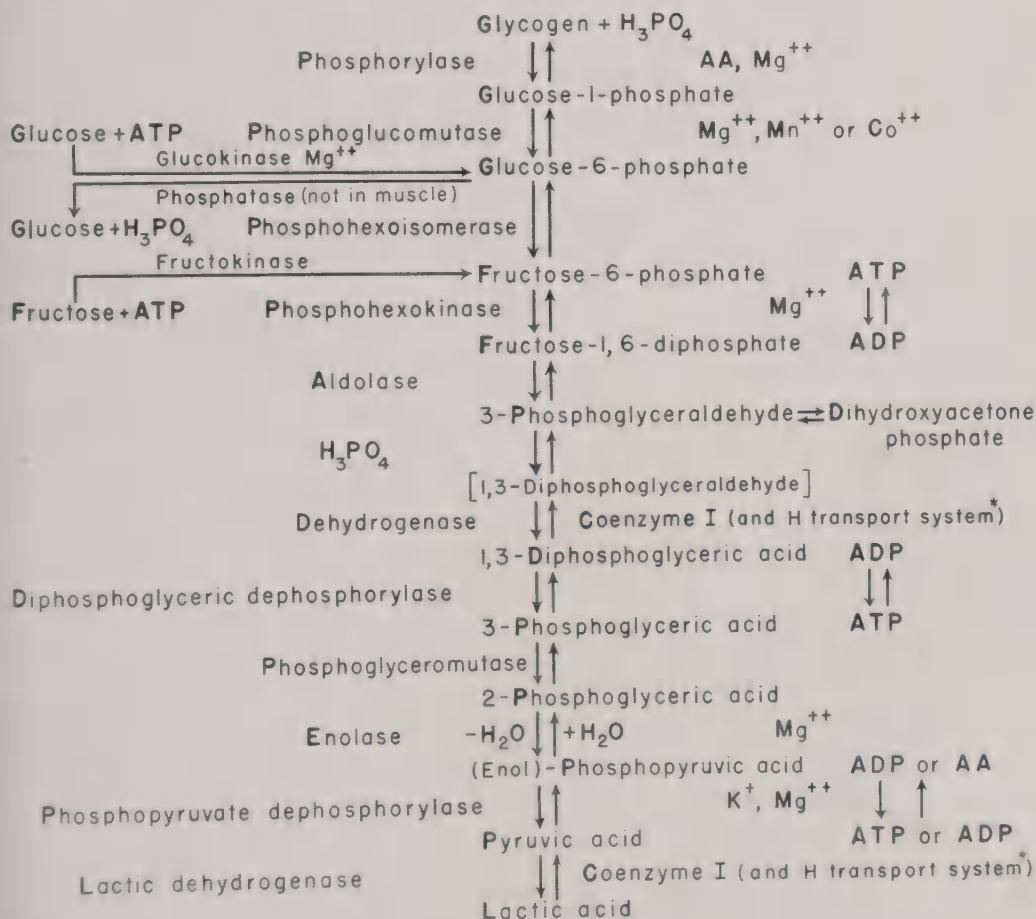
energy. It is this release of energy upon a suitable receptor in a muscle fiber which enables it to do biological work. Its energy-rich phosphate bonds can, however, be used to phosphorylate creatine, giving creatine an energy-rich phosphate bond; or it can phosphorylate glucose or fructose. In the latter case the energy is used in the process and the hexose-monophosphates formed do not possess energy-rich bonds. These transfers are shown in Fig. 53. In this figure creatine is also shown as acquiring its phosphate in the dephosphorylation of 1,3-diphosphoglyceric acid or enol-phosphopyruvic acid. This requires the intermediation of one of the adenosine phosphates. The adenosine phosphate may, of course, release or transfer its energy in some other way at this point.

In order to form energy-rich bonds again, phosphorylation must occur along with oxidations. It is the oxidations of the fragments of the carbohydrates which are useful in this respect. But first we must see how glycogen is broken down to these fragments.

The breakdown of glycogen follows, in general, this path:



This is shown in greater detail in Fig. 52.



\*See page 350.



The transformation of glycogen to hexose is a phosphorylation, and there are phosphate transfers at each step shown and at a number of intermediate ones not shown. At least ten enzymes take part in these transformations as well as a coenzyme and two phosphate carriers, namely, creatine phosphate and adenosine triphosphate. The latter is also called adenylic pyrophosphate. Several inorganic ions are also indispensable.

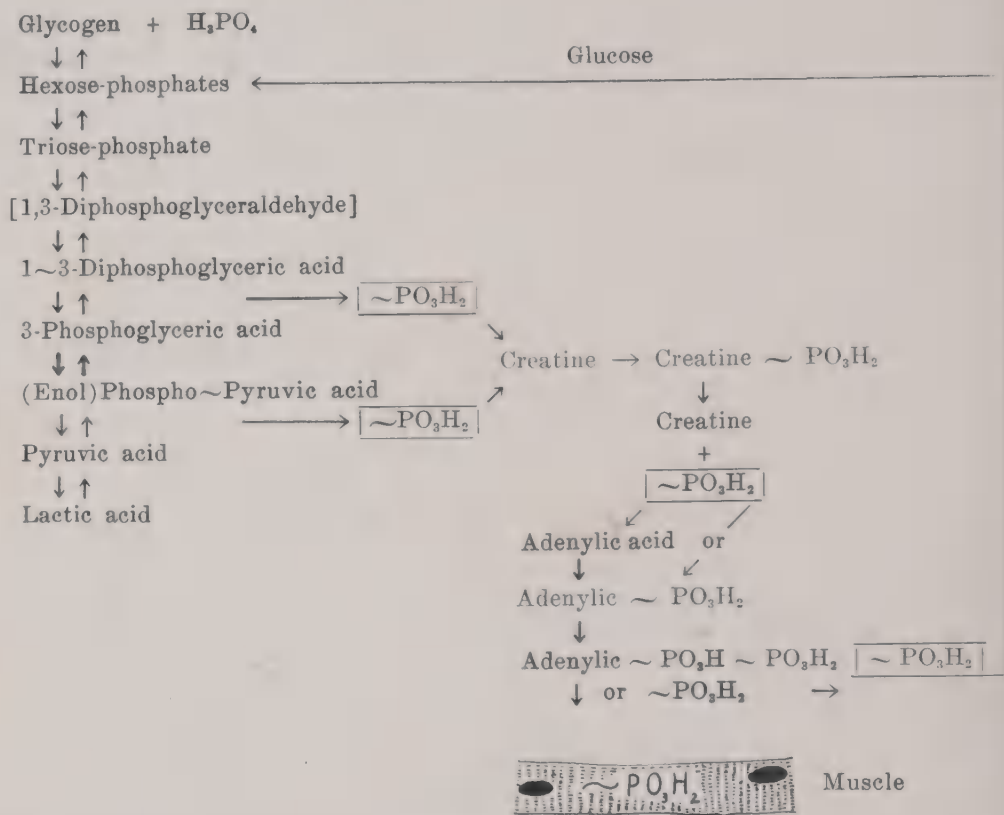
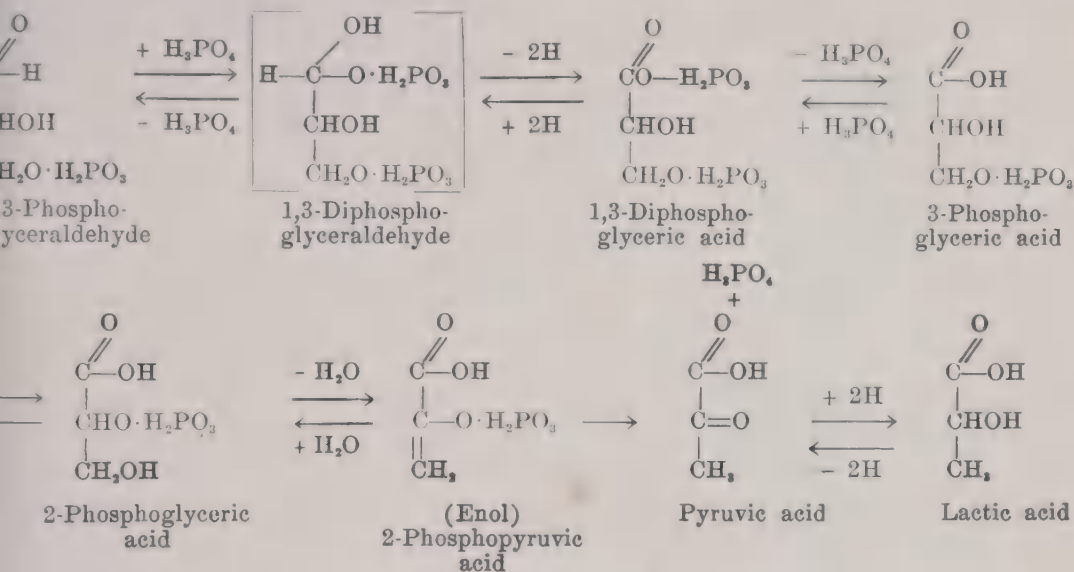


Fig. 53.—An outline of the possible relations of the various reactions in muscle contraction.

The first phase, glycogen  $\rightarrow$  hexose, really consists of several steps besides the initial phosphorylation. There is first formed glucose-1-phosphate; then the phosphate is shifted to the 6 position and the glucose is changed to fructose. Another phosphate is added to produce fructose-1,6-diphosphate. The fructose molecule is now split into two three-carbon chains of 3-phosphoglyceric aldehyde and dihydroxyacetone phosphate. The former is phosphorylated to the hypothetical di-phosphoglyceric aldehyde. The dehydrogenation of this yields diphosphoglyceric acid which is converted first to 3-phosphoglyceric acid and phosphate. This is a point at which energy is released. Next enol-phosphopyruvic acid is formed. Enol-phosphopyruvic acid adds on water to liberate pyruvic acid and phosphate and again there is a liberation of energy. Finally pyruvic acid is reduced by coenzyme I to yield lactic acid. Fig. 52 is an outline of this conception of glycogenolysis. In Fig. 53 the same reactions are given in an abbreviated form but the energy transfers are indicated.

Repeating the last part of Fig. 52, using structural formulas, we have:



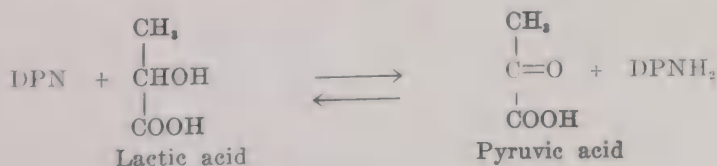
We may summarize these reactions as occurring in four steps.

1. Glycogen is phosphorylated and transformed into fructose-diphosphate.
2. Fructose-diphosphate is split into two three-carbon chains; i.e., two molecules of triose-phosphate.
3. In a series of transformations pyruvic acid is produced.
4. Pyruvic acid is reduced to lactic acid.

It is thus seen that lactic acid is the end product of glycogen decomposition during the anaerobic or contractile phase of muscle contraction. It is also evident that phosphate transfer plays an important role in these reactions.

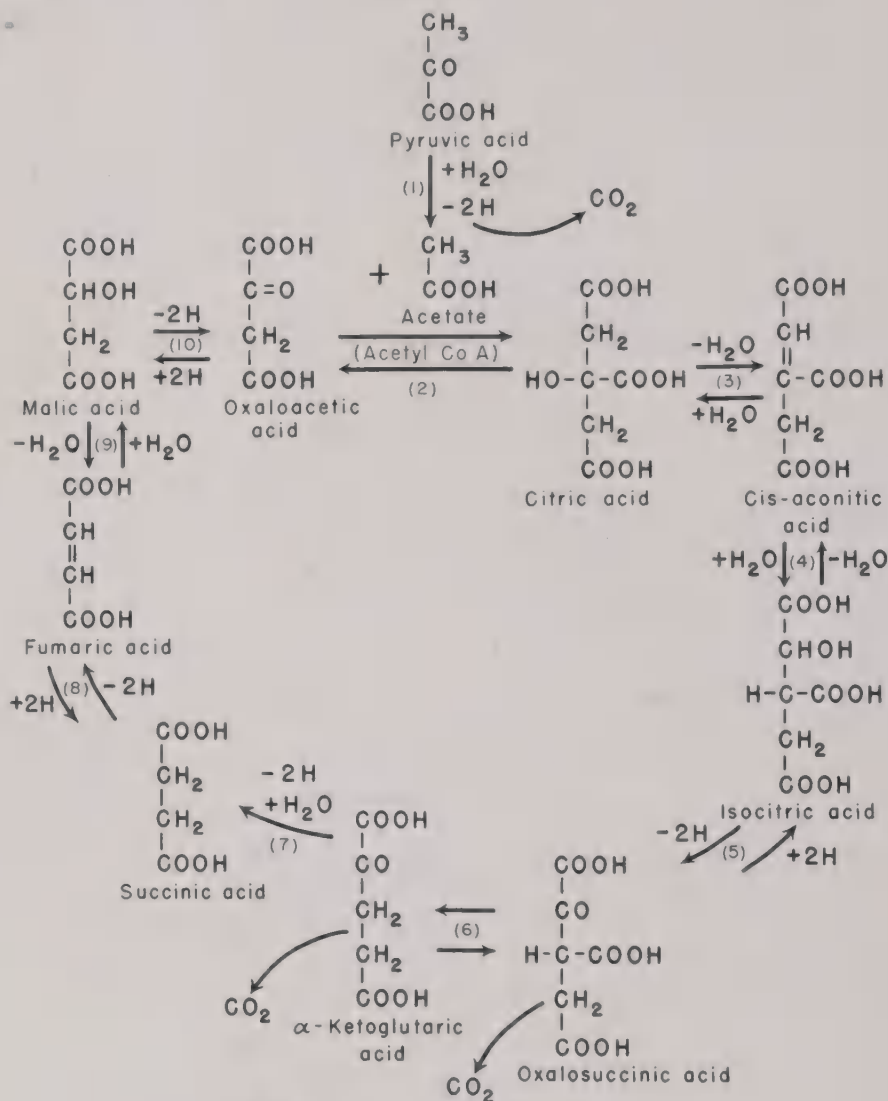
### Fate of Lactic Acid and of Pyruvic Acid

When there is active muscle contraction, the lactate of the blood is increased and this lactate is carried to the various cells throughout the body. Some of it is picked up by the liver and converted into glycogen. (Himwich.) The fate of the lactate in tissue cells depends upon the environmental conditions of the cell. If there is a diminished supply of oxygen, it is likely to go back through the steps which have been enumerated—back to glucose. However, in the presence of oxygen, reoxidation of lactic acid to pyruvic acid probably occurs. This involves lactic acid dehydrogenase and coenzyme I.



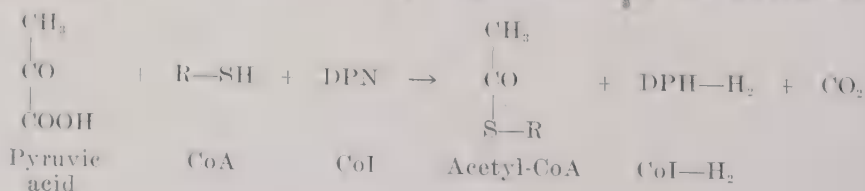
Pyruvic acid has been shown to be a normal intermediary in carbohydrate metabolism in man, and insulin seems to be a requisite for its formation (Bueding). It is a very reactive substance and its metabolism will depend

upon the oxygen supply, enzymes, pH, etc. There are various possibilities for the further oxidation of this compound, which may originate, not only from the oxidation of lactic acid but also in other metabolic reactions. One of the pathways is the Krebs, tricarboxylic acid, or citric acid cycle, which is given in simplified form below.



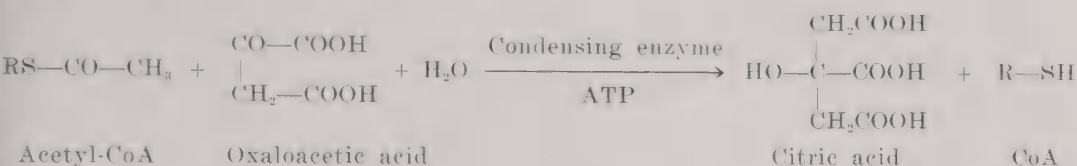
THE TRICARBOXYLIC ACID CYCLE  
(CITRIC ACID CYCLE)

Decarboxylation and oxidation of pyruvic acid constitute the first step and involve an enzyme, pyruvic dehydrogenase, together with DPN (coenzyme I), cocarboxylase, coenzyme A, magnesium ions, and phosphate.





Condensing enzyme then catalyzes the addition of the acetyl group of acetyl-CoA to oxaloacetic acid, but it should be noted that the methyl group is attached to the oxaloacetic acid; thus:



A series of reactions follows, resulting in the formation of three other tricarboxylic acids; namely, *cis*-aconitic, isocitric, and oxalosuccinic. The last named is decarboxylated, yielding  $\alpha$ -ketoglutaric acid and  $\text{CO}_2$ . Another  $\text{CO}_2$  is then released, producing succinic acid. A dehydrogenation yields fumaric acid, which is then hydrated to form malic acid. Another dehydrogenation results in oxaloacetic acid. In this manner oxaloacetic acid is, in a sense, regenerated so that it can combine with another molecule of acetic acid. This cycle thus continually oxidizes pyruvic acid. The enzymes, coenzymes, and activators involved, indicated by numbers on the diagram, are:

- (1) Pyruvic dehydrogenase; cocarboxylase, CoI, CoA, Mg.
- (2) Condensing enzyme; CoA, ATP
- (3) Aconitase
- (4) Aconitase
- (5) Isocitric dehydrogenase; CoII
- (6) Oxalosuccinic decarboxylase; Mn.
- (7)  $\alpha$ -Ketoglutaric oxidase; CoA, CoI
- (8) Succinic dehydrogenase
- (9) Fumarase
- (10) Malic dehydrogenase; CoI

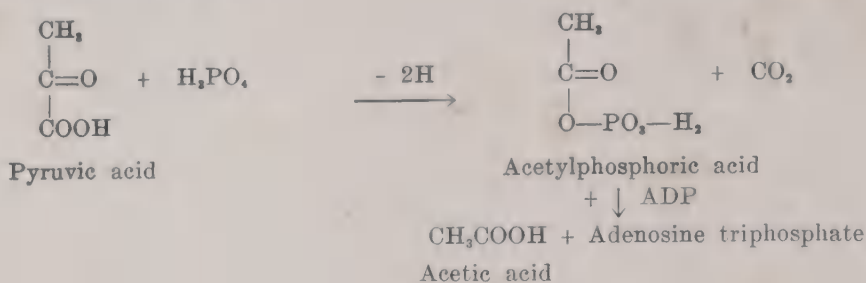
In this cycle it has been shown that the oxidation is coupled with phosphorylation and yields about sixteen high energy phosphate bonds. These arise as follows:

Pyruvate $\rightarrow$ Acetate + $\text{CO}_2$ yields	4 $\sim$ P bonds
Isocitrate $\rightarrow$ Oxalosuccinate yields	3 $\sim$ P bonds
$\alpha$ -Ketoglutarate $\rightarrow$ Succinate yields	4 $\sim$ P bonds
Succinate $\rightarrow$ Fumarate yields	2 $\sim$ P bonds
Malate $\rightarrow$ Oxaloacetate	3 $\sim$ P bonds

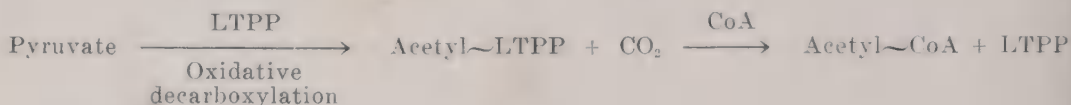
It is clear now how  $\text{CO}_2$  arises in biological oxidations. The three  $\text{CO}_2$  molecules, of course, are the oxidation products of the three C's of pyruvic acid, while the water comes from the oxidation of the H's. It will be shown subsequently how the products of protein and of lipid metabolism enter into the tricarboxylic acid cycle.

In nervous tissue (and probably in other tissues as well) pyruvic acid is oxidized to acetic acid or acetyl phosphate. This reaction is catalyzed by pyruvic dehydrogenase, and a number of factors are required, including riboflavin, as a hydrogen carrier, and a coenzyme, "co-carboxylase," which is now known to be thiamine-phosphate. In animals suffering from thiamine deficiency,

this reaction cannot proceed at a normal rate. Consequently an excess of pyruvic acid piles up and is probably the cause of the nervous affection. This oxidation of pyruvic acid is coupled with the phosphorylation of adenylic acid. The reactions are perhaps as follows:

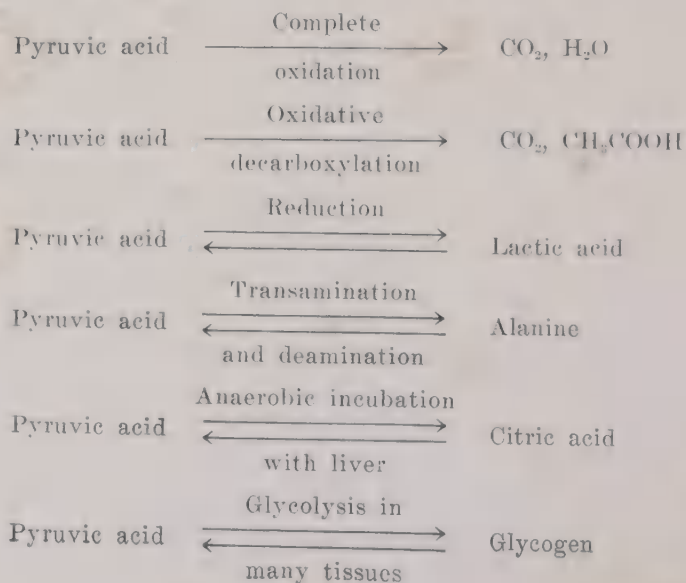


Pyruvic acid may undergo oxidative decarboxylation apart from its role in the tricarboxylic acid cycle. Lipoic acid and cocarboxylase (thiamine pyrophosphate) are probably concerned in the reactions involved. (See also page 306.) It is possible that lipoic acid conjugates with thiamine pyrophosphate to form lipothiamide pyrophosphate (LTPP), which is the active catalyst. The following chain of reactions has been suggested:



The reduced form of LTPP is reoxidized by diphosphopyridine nucleotide. (Reed and DeBusk.) Similarly  $\alpha$ -ketoglutarate is oxidatively decarboxylated to succinyl $\sim$ LTPP. It is probable that the acetyl $\sim$  or succinyl $\sim$ LTPP linkage occurs through a thiol bond. Energy transfer may occur through this bond as indicated.

Many other reactions occur in animal tissues in which pyruvic acid is a starting point. They may be summarized, in part, as follows:



There are undoubtedly other pathways for the catabolism of carbohydrate besides those described. It has been shown, for instance, that various mammalian tissues can oxidize carbohydrate readily when the anaerobic mechanisms are blocked by a specific inhibitor, iodoacetic acid. (Barker.) There are other studies which point in the same direction, that is, that the normal oxidation of carbohydrate is not limited to one or two mechanisms.

For example, there is the hypothesis that an aerobic oxidation of glucose-monophosphate might proceed through phosphogluconic acid  $\rightarrow$  2-keto-phosphogluconic acid  $\rightarrow$  arabinose phosphate. This involves a decarboxylation in the last step. By a repetition of these oxidations and decarboxylations, a four-carbon and finally a three-carbon sugar could result. There is some evidence for this scheme. (Lipmann; Dickens; Warburg and Christian.) It is also possible that aerobic oxidation of glucose might occur without phosphorylation. A D-glucose dehydrogenase has been shown to transform glucose into gluconic acid. Other carbohydrate derivatives, as well as pentoses, are oxidized by this enzyme, which may well be an alternate mechanism for the oxidation of sugar in mammalian liver. (Breusch; Wainio.)

**Muscular Contraction in the Intact Animal.**—It has been stated that isolated muscles can contract for a long time, but not indefinitely, in the absence of oxygen. Recovery, however, requires the presence of oxygen. The reasons for both of these phenomena must now be evident. The energy for contraction is derived from the dephosphorylation of creatine phosphate and adenosine di- and triphosphates, and from the breakdown of glycogen to lactic acid. These are not over-all oxidations. To rebuild them, however, energy is required and this is derived from the oxidation of lactic acid or pyruvic acid, or some related product.

In the intact animal a similar phenomenon occurs. During active muscular activity the same compounds, of course, are broken down to furnish the necessary energy and large amounts of lactic acid are formed in the process. The animal, or person, may not have enough oxygen available to oxidize this lactic acid while the contractions are going on. Recovery therefore must be postponed. The physiologists speak of this as an "oxygen debt." For example, for a 100-yard dash the oxygen requirement may be 6 liters. The maximum amount of oxygen which can be taken up is not over 4 liters per minute. Since this distance can be covered in about ten seconds, it is seen that the athlete can only take up a small fraction of the amount of oxygen he needs for the effort. Therefore, his respiration at the end of the dash is necessarily increased for quite a long time until the oxygen debt can be repaid. In light exercise there may be no oxygen debt. The amount of oxygen absorbed at the ordinary respiratory rate keeps pace with the amount of lactic acid which must be oxidized. This is called the steady state."

## CARBOHYDRATE METABOLISM IN HEART MUSCLE

Since the heart is a muscular organ which must contract almost constantly, one would expect to find there a rich store of glycogen. This is probably true, and the very low values reported in the literature are undoubtedly due to extremely rapid post-mortem glycogenolysis. The creatine content is less than half of the concentration of that compound in striated muscle. However, the



mechanism of carbohydrate metabolism in the heart is believed to be very similar to that described except that heart muscle apparently utilizes lactic acid to a greater extent. This may indicate a different metabolic pathway at one stage. Creatine phosphate and adenosine triphosphate play important roles here as they do in striated muscle contractions. The left ventricle contains higher concentrations of creatine, phosphorus, potassium, and adenine than the right. This means that creatine phosphate and adenosine triphosphate, as the dipotassium salts, are found in greater concentration in the stronger muscle. Furthermore, when cardiac hypertrophy begins, a slight increase in these constituents is seen, but with further hypertrophy they fall and reach their lowest values with extreme cardiac hypertrophy and heart failure. This points to the great need of creatine phosphate and adenosine triphosphate for efficient heart muscle action (Myers).

### CARBOHYDRATE METABOLISM IN NERVOUS TISSUE

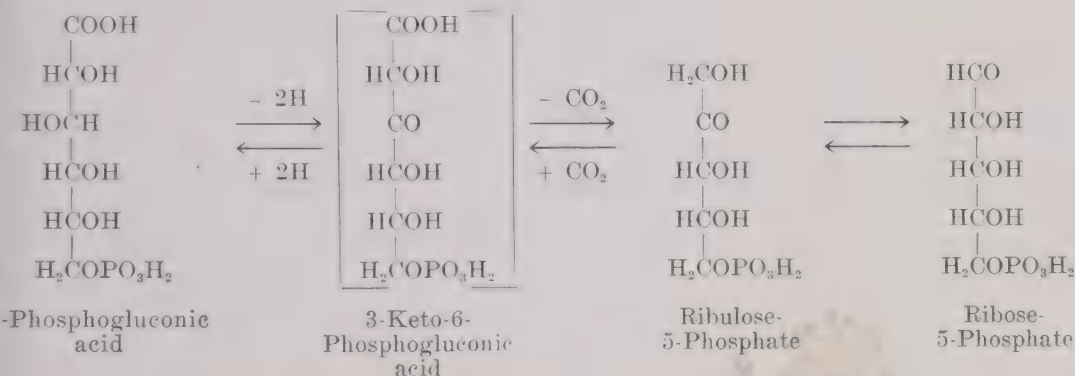
From the studies of Himwich and Nahum it appears that, in the brain of dogs, carbohydrate, or a derivative, is the sole source of energy. The respiratory quotient was close to 1.0, which is the respiratory quotient for carbohydrate (see page 556). Furthermore, the blood coming from the brain contained less glucose and lactic acid than that going to it. In nerves at rest the respiratory quotient indicated combustion of a mixture of protein, carbohydrate, and fat, but during activity the respiratory quotient rose to such an extent that it indicated that the extra metabolism was entirely derived from carbohydrate. There is very little glycogen present in brain, and it is improbable that there is much glycogenesis or glycogenolysis there. Glucose is the principal carbohydrate used by nervous tissue, and the exact path of decomposition is unknown. Under anaerobic conditions, brain tissue transforms glucose into lactic acid, but if oxygen is present, the amount of lactic acid formed is greatly diminished. This may mean that lactic acid is always formed from glucose but in the presence of oxygen it is immediately oxidized and thus removed. As mentioned before, pyruvic acid increases in nervous tissue in the absence of vitamin B<sub>1</sub>. This would indicate that it is a normal intermediary in glucose utilization in nervous tissue.

We know that the brain requires both oxygen and glucose. If glucose or lactic acid is added to chopped brain tissue, there results an increased oxygen consumption (Quastel). Evidently brain tissue takes up oxygen for carbohydrate utilization. When a hypoglycemia occurs, one of the striking symptoms is its effect upon the brain. There is a mental confusion, dizziness, and sometimes delirium. This may occur after an overdose of insulin. In this connection the recent use of insulin-induced hypoglycemia in the treatment of schizophrenia may be pointed out.

### METABOLISM OF PENTOSES

The pentoses of the food are not phosphorylated in the intestinal tract and consequently are not readily absorbed. They are not utilized as well as the hexoses when given by mouth. However, it is possible that in physiologi-

al combinations they are readily metabolized since it appears that the enzymes of various tissues can change the ribose group of purine nucleotides and nucleosides to some nonpentose form, probably a hexose. (Schlenk and Waldvogel.) Another pentose, L-xyloketose, may be synthesized by the body by way of glucuronic acid in the condition known as pentosuria. (Enklewitz and Tasker.) It is unavailable to the body for metabolic purposes and for this reason is excreted. Pentoses arise in the body from hexoses, through gluconic acid. The enzyme system, which accomplishes this reaction, occurs not only in yeast, where it was first discovered (Scott and Cohen), but also in the liver and bone marrow (Seegmuller and Horecker). Two interesting features of the reaction are a  $\beta$ -oxidation and the presence of a phosphopentose isomerase, which catalyzes the reversible conversion of D-ribulose-5-phosphate to D-ribose-5-phosphate. The following scheme represents the reactions:



The reversibility of this reaction has been demonstrated by the fixation of  $^{14}\text{O}_2$  in 6-phosphogluconate. (Horecker and Smyrniotis.)

## ABNORMAL CARBOHYDRATE METABOLISM

An understanding of many of the phases of normal carbohydrate metabolism has come from a study of pathological or experimental derangements of the normal. Most of these result in glucosuria. Glucosuria is merely a symptom of a metabolic disorder. The sugar appears in the urine either because the blood sugar is too high or the renal threshold is too low, and the real problem is why does either of these occur. The first to be considered is alimentary glucosuria. This is easy to understand. Large amounts of carbohydrate are available in the gastrointestinal tract and are absorbed more rapidly than they can be assimilated by the normal processes. The blood sugar rises above the renal threshold and glucosuria results (see page 524). Individuals, however, vary in their power to utilize excessive quantities of carbohydrates, due in all probability to differences in their endocrine balance. Consequently alimentary glucosuria may be more easily provoked in some people than in others.

The piqure of Claude Bernard, i.e., an injury to the floor of the fourth ventricle, is an experimental method of inducing hyperglycemia with consequent glucosuria. It is probably a stimulation of the adrenal medulla, via the splanchnic nerves, causing an increased secretion of adrenaline, which is

apparently the effective factor. Direct stimulation of the great splanchnic nerves or injection of adrenaline will have the same result. The adrenaline in some way increases glycogenolysis in the liver and the blood sugar rises. It has a similar effect upon muscle glycogen. All of these are temporary effects.

When phlorizin is administered to animals, glucosuria results. This was discovered by von Mering and has been developed and used in this country by Lusk, and others. The glucosuria continues as long as the drug is given, with results such as would be expected from a continued loss of sugar. The condition differs from true diabetes mellitus in that the glycemia is either normal or slightly below normal. The explanation is that phlorizin interferes with the phosphorylation of glucose. The glomerulus permits glucose to filter through it into the tubules, from which normally it is reabsorbed. This reabsorption depends upon phosphorylation and if phosphorylation is blocked, as by phlorizin, the glucose flows through the tubules into the urine. Thus, in phlorizin diabetes we have a low renal threshold due to a failure in phosphorylation of glucose.

Nonhyperglycemic glucosuria occurs in man occasionally. Such a condition is "renal diabetes" or renal glucosuria, a rather rare state. The blood sugar is normal or below normal. Sugar is present in the urine at all times and is almost independent of the amount of carbohydrate ingested. There is no apparent disturbance of carbohydrate metabolism. This is evidenced by a normal respiratory quotient and no change in fat metabolism. It apparently never develops into diabetes mellitus and seems to be a harmless malady. The cause is unknown, but one is tempted to ascribe it to a slight difficulty in phosphorylation in the kidney tubules.

In a fairly large percentage of normal pregnant women glucosuria occurs. This is not a lactosuria, which is more likely to be found during the period of lactation. It is said to be due to a decreased carbohydrate tolerance resulting from physiological hypertrophy of the pituitary gland which occurs during pregnancy. The blood sugar does not rise much above normal.

Certain kidney conditions are frequently associated with glucosuria. In some instances hyperglycemia occurs and in some the blood sugar is normal. In glomerulonephritis and nephrosclerosis, glucosuria with either a high or normal blood sugar may be encountered. In nephrosis there is glucosuria with no increase in the blood sugar. The low blood sugar values in all of these renal conditions are explained on the basis of degenerative changes in the renal tubular epithelium. As a result of these, there is a physiological inability to reabsorb the sugar; i.e., the renal threshold is lowered. No good explanation for the hyperglycemias in these cases is available.

Glucosuria with hyperglycemia is known as diabetes mellitus, when it occurs in man. Among the symptoms of severe cases are excessive thirst and polyuria. If uncontrolled, there is muscular weakness, and acidosis, with the possibility of diabetic coma and death. The excessive thirst and polyuria are due to the large amount of water needed to dissolve the glucose as it is excreted by the kidneys. The weakness is mostly due to the inability to utilize the requisite amount of food and often to dehydration due to the polyuria. The



acidosis and coma result from a disturbance in fat metabolism which takes place concurrently with the disturbance in carbohydrate metabolism. Another effect is upon protein metabolism. If the malady is not controlled, the patient goes into negative nitrogen balance. Thus diabetes is a disturbance of carbohydrate, fat, and protein metabolism.

For many years there was a suspicion that the pancreas was in some way related to diabetes. There was no proof of this until 1889-1891, when the physicians von Mering and Minkowski succeeded in completely removing the pancreas of a dog surgically. This was an exceptionally difficult feat since the pancreas is quite adherent to the duodenum. Moreover the healing of the abdominal wound is difficult in the absence of the internal secretion of the pancreas. However they did accomplish it and thereby ushered in a new era in the study of carbohydrate metabolism in general and diabetes mellitus in particular. In these depancreatized dogs developed a pathological state which resembled very severe human diabetes mellitus. They had hyperglycemia and glycosuria, became exceedingly thirsty and hungry, and passed large volumes of urine. At a time they grew weak and thin, had acetone bodies in blood and urine, and died in coma in from two to six weeks. If, instead of removing the pancreas, it was carried, with nerves and blood vessels intact, outside of the peritoneal cavity and left under the skin, diabetes did not result. This proved that the external secretion, that is, the pancreatic juice, was not the factor involved in preventing diabetes. Subsequent removal of the transplanted pancreas caused glycosuria to occur. Following the work of these pioneers, many experimenters attempted to determine the mechanism of this action. The explanation advanced by Lépine was that the pancreas produces an internal secretion (besides its external digestive secretion) which is poured directly into the blood stream and in some way regulates carbohydrate metabolism. Hédon, Gley, and a number of other investigators planned various cross transfusions between diabetic and normal animals, and in vitro tissue and blood studies. While not decisive, they indicated that the pancreas has an internal secretion which is effective only in the living animal. Carlson depancreatized a pregnant bitch and found that she did not become diabetic until after the pups were born. The fetal pancreases had protected the mother by their internal secretion.

The crucial experiment of injecting pancreatic extracts into diabetic animals was attempted many times, but the early work was indecisive. In 1908 Zuelzer prepared an alcoholic extract and noted reductions in the output of urinary sugar and acetone bodies in a diabetic dog and in several patients. However, a decrease in urinary sugar is not a satisfactory criterion of improvement in this condition since it may result from a diminished intake of food, due to a loss of appetite resulting from treatment, or to an influence on the kidneys. Consequently, although Zuelzer probably was using a potent pancreatic extract, his work was not accepted or appreciated. E. L. Scott, in this country, used water extracts of pancreatic tissue that had previously been extracted with alcohol. Upon injection into diabetic dogs the sugar excretion was diminished and the G:N ratio was also lowered (see page 375). This indicated that some of the glucose presumably arising from protein metabolism was being utilized, and this marked

a distinct advance. In 1915 Kleiner and Meltzer showed that a simple aqueous "emulsion" of pancreatic tissue, when injected very slowly intravenously into diabetic dogs, had an almost immediate effect in lowering the blood sugar and, of course, the excretion of glucose. This was considered definite evidence that the pancreas has an internal secretion which can be obtained from it by appropriate extraction methods, and they suggested that it might have a possible therapeutic application.

## INSULIN

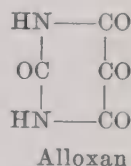
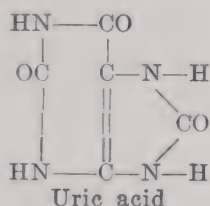
The climax of these many series of experiments came in 1922. Banting and Best, working in Macleod's laboratory, conducted some brilliant researches which led to the discovery of insulin. The pancreas contains two types of cells, the acinar or glandular cells, which secrete the pancreatic juice and make up the bulk of pancreatic tissue, and groups of small, irregular, polygonal cells. The latter were discovered by Langerhans in 1867 and have been called the "islands of Langerhans," or simply "islet tissue." It was suspected that here was the site of the manufacture of the internal secretion of the pancreas. In fact D'Arnozan and Viacard had shown that if the pancreatic ducts were tied off, so that pancreatic juice could not flow into the duodenum, the acinar cells degenerate and disappear and nothing is left of the pancreas except islet tissue. Such animals do not develop diabetes. The idea occurred to Banting, "Ligate pancreatic ducts of dogs. Wait six to eight weeks for degeneration. Remove the residue and extract." After considerable difficulty, Banting and Best were able to perform this double experiment with results which bore out his expectations.

The extract prepared from such islet tissue proved to have a very potent effect in reducing the hyperglycemia of diabetic dogs and then, after careful purification, it was found to have similar effects upon diabetic human beings. A large group of investigators was then organized to work out the various phases of the problem. Among them were Collip, Campbell, Fletcher, and Noble. The best sources and methods of extracting, purifying, and concentrating insulin were sought. It had been known that in certain teleostean fishes the islands of Langerhans exist as organs separate from the acinar pancreatic cells. These furnished extracts having the same properties as those obtained from mammals. This gave added proof that the islands of Langerhans were the source of insulin. Beef and hog pancreas are the commercial sources of insulin at present, and several methods of preparation have been devised. Insulin is soluble in water, in alcohol of a strength up to about 80 per cent, in acids, and alkalis. However, it is not stable in alkaline solution. Collip's method employs the principle of fractional precipitation with alcohol. Doisy, Somogyi, and Shaffer made use of the fact that insulin is least soluble at its isoelectric point, pI 5.3, and that impurities may be precipitated out of solution and thus be removed at pHs above or below pH 5.3. From preparations of insulin used therapeutically Abel and his collaborators obtained insulin in two different crystalline forms. However, it is undoubtedly one substance. It is a protein with a molecular weight of about 6,000. Its amino acid composition and structure have been



described on page 116. Cystine occurs in large amounts and there is some evidence that the S-S group is connected with its physiological activity. Zinc is always found with this hormone, but it is not a part of the insulin molecule. The crystals are probably the zinc salt of insulin. Since it is a protein, it is not surprising that it is digested and thus inactivated by the proteolytic enzymes pepsin and trypsin. Hence insulin has no effect when taken orally and must be administered parenterally. This constitutes one of the chief difficulties and objections to its use.

**Alloxan Diabetes.**—The injection of alloxan into animals is a chemical method of producing diabetes. It causes an initial hyperglycemia which is apparently due to a stimulation of the adrenal medulla. (Goldner and Gomori.) A transitory hypoglycemia then is seen, probably a result of liberation of insulin from the beta cells of the pancreas. (Jacobs.) If glucose is now given to feed the animal over the hypoglycemic phase, hyperglycemia and other diabetic symptoms ensue. (Dunn.) The final effect is a necrosis of the beta cells which produces the permanent diabetes. Alloxan is an oxidative product of uric acid:



The diabetes of these animals is characterized by very severe glycosuria with a high insulin requirement. However, they have a low ketone body excretion and if untreated with insulin live longer than depancreatized dogs under similar conditions. This is related to the fact that there are two types of cells in the islands of Langerhans, the  $\alpha$  and  $\beta$  cells, and their different functions were strikingly shown by some experiments of Thorogood and Zimmerman. Dogs were made diabetic by the administration of alloxan, which destroyed their  $\beta$  cells, and their insulin requirement was determined. They were then depancreatized, thus losing their  $\alpha$  cells as well as their  $\beta$  cells. The insulin requirement was now found to be much less than before. The interpretation is as follows: Insulin is produced by the  $\beta$  cells, and another hormone, glucagon, by the  $\alpha$  cells. The latter hormone opposes the action of insulin, increasing blood sugar, and may also prevent the formation of excessive amounts of ketone bodies. Consequently an alloxan-diabetic dog not only lacks insulin because it lacks the  $\beta$  cells, but it possesses a factor which still further acts in the same way that a deficiency of insulin does. Therefore its blood sugar is higher than that of a depancreatized dog. Alloxan diabetes may be prevented from occurring if glutathione or cysteine is given along with the alloxan. It is possible that cysteine and glutathione reduce alloxan to dialuric acid, a substance which presumably has no diabetogenic effect. On the other hand, alloxan inactivates coenzyme A, which, like cysteine and glutathione, has an -SH group. It is thought that the diabetogenic action of



alloxan may, in part, be due to the inactivation of coenzyme A within the  $\beta$  cells of the islands of Langerhans, thus destroying those cells. (Lazarow.)

Whether these observations have any bearing upon the etiology of the disease in man is still a matter of speculation, but it is interesting to note that the presence of alloxan in normal liver tissue has been reported. (Ruben and Tipson.)

Glucagon has been isolated and crystallized. (Staub.) It is a protein but is quite different from insulin in its crystalline form and in having a low content of sulfur. The small amount of sulfur present is in the form of methionine rather than cystine. The pancreas of birds is very rich in this factor, and, since there is also present in birds' pancreas a great number of cells resembling alpha cells, this lends support to the concept that glucagon is formed by the alpha cells of the pancreatic islets. (Vuylsteke and De Duve.) It should also be noted that the action of glucagon is manifested in the brief period of hyperglycemia which occurs after commercial insulin is injected intravenously into animals with adequate stores of liver glycogen. In fasted animals it has no effect. It thus appears to be a glycogenolytic agent but not a gluconeogenetic one. It is believed to act upon the same enzyme system as epinephrine. Glucagon has no hyperglycemic effect after subcutaneous injection, perhaps because proteases present in the tissue fluids inactivate it. It is an "impurity" present in most brands of insulin.

In this connection, it may be of interest to note that dehydroascorbic acid, dehydroisascorbic acid, and dehydroglucoascorbic acid are all diabetogenic (Patterson), and the explanation offered is that they act by destroying the sulfhydryl groups of enzymes essential to normal carbohydrate utilization. Evidently  $-SH$  and  $-S-S-$  groups are quite important in carbohydrate metabolism. In fact, Martinez has shown that the  $SH$  content of blood and tissues falls in depancreatized animals and that pretreatment with sulfhydryl compounds decreases the severity of the resultant diabetes. Among other compounds which have been shown to cause diabetes experimentally are  $\beta$ -hydroxybutyric acid and other intermediary products of fat metabolism. (Nath and Brahmachari.)

### Action of Insulin

When injected into a normal animal (the rabbit is usually the species employed) the blood sugar falls to very low levels—from 30 to 50 mg. per cent. "True" blood sugar (that is reduction due to glucose, apart from that due to nonsugars) is even lower, and may, indeed, fall to almost zero. Frequently convulsions ensue. Sometimes these have a fatal outcome. They can be combated if glucose is given soon after they begin. The effect of glucose under these circumstances is astonishing. In a very few minutes the animal, which has been lying on its side having violent convulsive movements, rights itself and hops around in a perfectly normal manner. This effect of insulin upon the rabbit is utilized in standardizing commercial preparations. (See page 603.)

In diabetic animals and human beings the effect of insulin is the same. It lowers blood sugar and thereby diminishes glycosuria. If ketonemia and acidosis are present, it tends to combat these conditions also. The effect, however, is

porary; the blood sugar eventually rises again and insulin must be administered at least once a day in order to keep the blood sugar at a normal level. The decrease in blood sugar is accompanied by glycogenesis in both liver and muscle. There is an increased consumption of oxygen and a consequent rise in respiratory quotient which indicates the utilization of glucose by the tissues. There is an inhibition of gluconeogenesis (see page 417); i.e., the formation of glucose from proteins and fats. Lastly, insulin promotes the deposition of fat.

In normal animals, apparently no increase in liver glycogen occurs (there may even be a decrease), but there is an accelerated absorption of oxygen and increased glycogenesis in muscle. The explanation is as follows: Any insulin administered to a normal animal or person is an excess over the optimal amount already present. It prevents liver glycogenolysis at first and causes hypoglycemia. The blood sugar "lost" is deposited in the muscles as muscle glycogen. Now the hypoglycemia paradoxically opposes the effect of insulin on the hepatic glycogen because hypoglycemia causes the secretion of adrenaline. This is a normal physiological result of hypoglycemia and is an emergency mechanism resulting in the adrenaline-glycogenolysis action. That the effect of insulin here is to produce hypoglycemia; hypoglycemia causes the production of adrenaline; adrenaline results in glycogenolysis intended to counteract hypoglycemia. The sugar thus released is deposited in the muscles as glycogen and the blood sugar remains low. Therefore we find that the glycogen reserves of the liver are either not increased or are diminished after insulin administration to a normal animal.

**Glucose Tolerance Tests.**—A surprisingly large amount of glucose may be absorbed by a normal person without any being excreted in the urine. At the same time there is only a moderate rise of blood sugar and this for only a short time. If diabetes, even in the mildest degree, or renal diabetes, or certain other endocrine or renal disturbances exist, there is very likely to be glucosuria and an entirely different blood sugar picture. By standardizing the procedure "glucose tolerance test" results and more or less characteristic blood sugar curves are obtained. A number of different methods are used. The amount of glucose may be from 1.5 to 1.75 Gm. per kilogram of body weight in a 10 per cent solution, flavored with lemon juice. Some workers favor other amounts; still others use a standard meal containing protein, carbohydrate, and fat. The blood is taken before the glucose is ingested and at regular intervals thereafter. Again, there is not complete agreement as to the number of samples to be withdrawn or the intervals between sampling. Perhaps the most generally adopted plan is to take one sample at the half hour, then at the end of the first, second, and third hours. A normal curve is one which reaches maximum (seldom over 160 mg.) at or before the end of the first hour, with return to normal by the end of the second, or, at most, the third hour. Diminished tolerance is indicated by a more marked rise and a slower return to normal. The urine should be collected at about the same time each blood sample is taken. Normally there is little or no sugar excreted. A normal or normal curve with glucosuria is evidence of renal diabetes. A high curve (diminished tolerance), if accompanied by glucosuria, indicates diabetes mellitus,

or a prediabetic state if the curve is not greatly accentuated. A high curve with no glucosuria may mean a renal condition. Typical curves are shown in Fig. 54.

In the treatment of diabetes mellitus in human beings the physician must estimate the correct amount of insulin to be given to enable the patient to utilize the carbohydrate needed for his activities. This will vary with the severity of the disease and the energy requirements of the patient. The medication is given about a half hour before a meal in order to allow for proper absorption and distribution. An overdose is likely to have very unfortunate results. At first there may be hunger or a feeling of nervousness. This is followed by perspiration, alternate pallor and flushing, and dizziness. There may even be delirium, stupor, and convulsions. Many individuals who take insulin regularly wear identification tags stating that they are likely to have hypoglycemic symptoms and giving instructions as to the treatment to be given

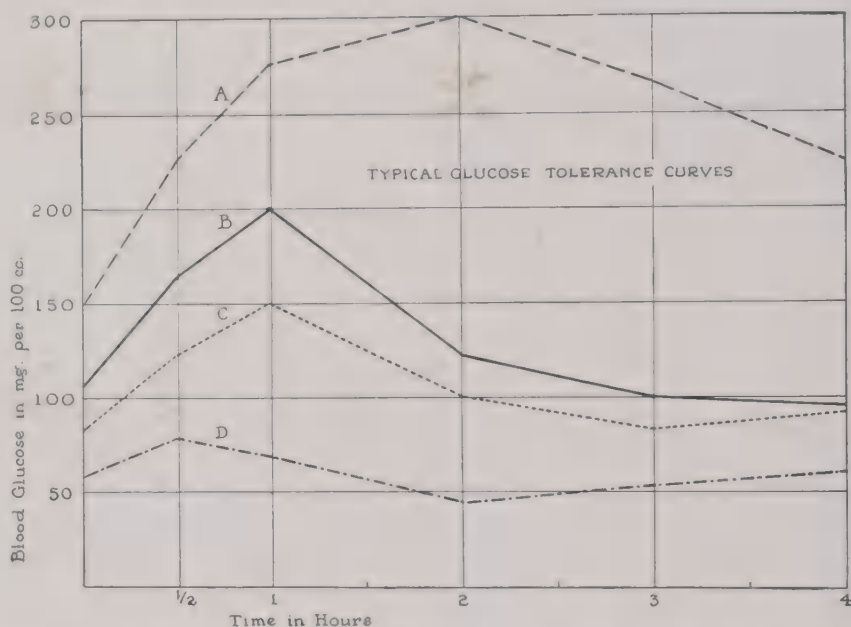


Fig. 54.—Typical glucose tolerance curves. A, Diabetes mellitus; B, hyperthyroidism; C, normal; D, Addison's disease, or hypothyroidism, or hyperinsulinism. (From Andes, J. E., and Eaton, A. G.: *Synopsis of Applied Pathological Chemistry*, St. Louis, 1941, The C. V. Mosby Co.)

them. A diabetic patient suffering from insulin shock should receive glucose intravenously but no additional insulin, whereas one in diabetic coma should be given insulin. Some diabetic patients can recognize the premonitory symptoms, whereupon they take some sugar or candy which it is advisable for them to have with them at all times. Because of the inconveniences and possible dangers in the use of insulin, mild cases are almost always treated by dietary regulation alone.

Since the effects of insulin wear off rather rapidly, several new preparations are now available which are absorbed slowly and therefore act over a longer period. The first of these was produced by combining protamine with insulin forming a sort of conjugated protein, protamine-insulin (Hagedorn and associates). The addition of zinc to this gave protamine-zinc-insulin, which is still



are slowly absorbed. Globin-insulin is a compound of globin, derived from hemoglobin, and insulin. This also contains added zinc. The onset of its effect is more rapid than protamine-zinc-insulin but not quite as rapid as regular insulin. Its hypoglycemic action is not as prolonged as that of the protamine-zinc-insulin. In Fig. 55 are shown the general types of blood sugar curves produced by the latter two in comparison with "regular" insulin. A new type of protamine insulin is called NPII (Neutral Protamine Hagedorn). Its action resembles that of globin insulin.

In the usual treatment of a diabetic patient there is a nice adjustment of diet and the insulin dosage. Often a mixture of regular and slow-acting insulin is prescribed, the regular for a quick effect and the slow-acting to maintain this effect. In severe cases the administration of insulin may have to be repeated in the course of the day. The total caloric and nitrogen requirements are calculated and a diet carefully planned. There are several types

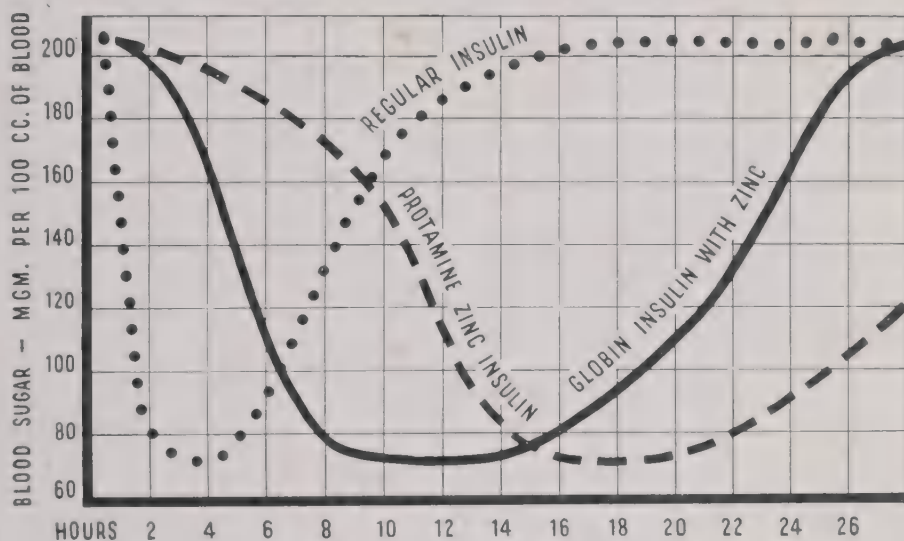


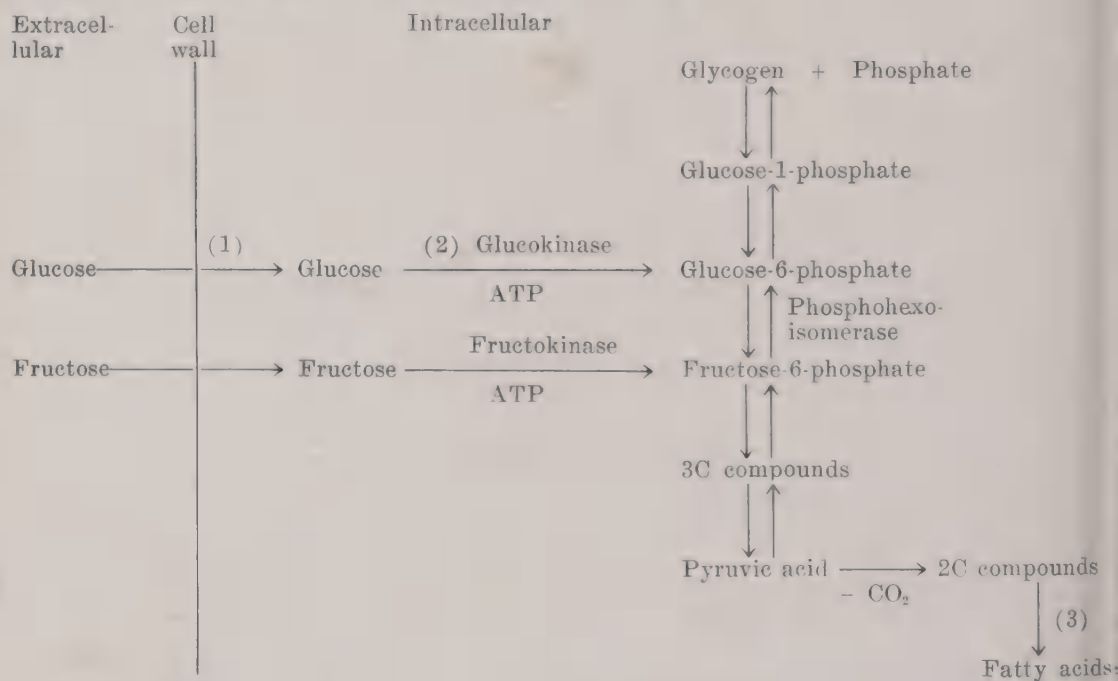
Fig. 55.—General types of blood sugar curves following the administration of regular insulin, protamine-zinc-insulin, and globin-insulin. (Courtesy Dr. Donald D. Searle of Burroughs Wellcome & Co., Inc.)

diet advocated by various authorities. (See page 336.) The type, dosage, and timing of the insulin administration will vary with the individual patient and with the experience of the physician. The aim is to keep the blood sugar as near normal as possible, in order to accomplish the maximum utilization of sugar, and also to prevent hyperglycemia, which is believed, by some physicians, to have a harmful effect upon tissues. Another type of treatment could also be presented. This is called "clinical control," as contrasted with "chemical control," outlined above. Clinical control has the objectives of (1) elimination of the symptoms of the disease, (2) maintenance of or gain in weight, and (3) the avoidance of ketonemia. Hyperglycemia and glucosuria are disregarded as long as no diabetic symptoms are present. The diet is not carefully computed; indeed the patient is allowed an almost normal diet. One daily dose of slow-acting insulin is given. The amount of insulin used will

depend upon the severity of the condition. The theory is that the insulin should permit of a sufficient utilization of carbohydrate for ordinary needs, and the excess in the blood does no harm. (Tolstoi.)

An approach from the nutritional side may also be mentioned. A study of a large number of diabetics by Biskind and Schreier gave evidence of some deficiency of the B vitamins in every case. They also point out that in diabetes there is an impairment in hepatic function. Therefore they advocate the administration of fairly large amounts of B vitamins and liver extract and state that blood sugar levels and insulin requirements are usually reduced.

**Mechanism of Insulin Action.**—On the accompanying diagram are shown the points where some of the actions of insulin have been postulated.



The first clue to the exact mechanism of insulin action was the discovery of its effect in the hexokinase, or glucokinase, reaction. Glucokinase is the enzyme which catalyzes the phosphorylation of glucose. Adenosine triphosphate is the coenzyme or phosphate donor in this reaction. The formation of glucose-6-phosphate is a prerequisite for any further step in the utilization of glucose. Study of Fig. 52 will demonstrate its key position both for the formation of glycogen from glucose and for the oxidation of glucose, whether it is derived from glycogen or from food. It is inhibited by anterior pituitary extract, and this inhibition is abolished by insulin. (Price.) Thus far no direct effect of insulin alone upon the reaction has been discovered, but only the indirect one described. However, it seems probable that insulin is required for the optimum production of the first phase of glucose utilization; that is, insulin increases the rate of phosphorylation, and thus the rate of glycogenesis. This point is indicated by (2). The similar phosphorylation of

fructose is not influenced by insulin. Therefore it is now believed that fructose can be utilized better than glucose by diabetics. However, neither glucose nor fructose is converted to fatty acids by diabetic liver slices, although this is accomplished by normal liver tissue. Insulin enables the diabetic tissue to make this conversion. This metabolic block has been localized at the point (3) where a two-carbon degradation product of the sugars is utilized in the formation of fatty acids, perhaps via the tricarboxylic acid cycle.

Another hypothesis involves the first step in the utilization of glucose. (Levine.) This states that insulin facilitates the passage of glucose into the cell, independently of the glucokinase reaction. Whether or not this is an effect upon the permeability of the cell membrane (1) is not definitely stated. Skeletal muscle does not have any galactokinase activity, but insulin facilitates the rate of distribution of D-galactose and does the same for L-arabinose and for D-xylose. These three sugars, the only ones showing the same effect, have the same configuration about the first three carbons as D-glucose has. (See pages 48 and 49.) Stadie has shown that insulin is combined at the surface of intact muscle fibers, a prerequisite for its action. It is possible that this fixation of insulin may be related in some way to the phenomenon just described.

In harmony with the direct observation of an influence of insulin upon phosphorylation is the fact that insulin administration is followed by a lowering of the blood phosphate, indicating the participation of phosphate in sugar utilization. (Lundsgaard.) This hormone also restored the ability of heart muscle to synthesize phosphocreatine, when this ability had been diminished by alloxan diabetes. (Goranson and Erulkar.) Insulin has been shown to have beneficial effects in carbohydrate metabolism in a number of *in vitro* experiments. For example, it increased the amount of pyruvate metabolized to  $\text{CO}_2$  by diabetic muscle. This was ascertained by using pyruvate labeled with  $\text{C}^{14}$ . (Villie and Hastings.) It also stimulated the synthesis of fatty acids from acetate when pyruvate was also present. (Bloch and Kramer.) Furthermore, insulin has an effect on protein metabolism. It appears to be required for the nitrogen storage action of the growth hormone. The latter causes the secretion of extra insulin. The extra insulin then has a synergistic action with the growth hormone on protein anabolism. (Lukens.)

**Hypoglycemia.**—Before the advent of insulin, little was known of hypoglycemic conditions. The stimulation of the study of carbohydrate metabolism which this discovery brought about, and particularly the greatly increased number of blood sugar determinations which became almost routine hospital procedure, led to the realization that hypoglycemia is not a rare condition. Proliferation of islet tissue, i.e., tumor of the pancreas, frequently occurs; the increase in insulin secretion causes hypoglycemia. Administration of glucose tends to ameliorate the symptoms and surgical removal often has a permanent beneficial result. Hypoglycemia also may be caused by a diminution in the secretion of those glands of internal secretion which have an opposite effect to the glands of Langerhans; namely, the anterior pituitary, the thyroid, and either the cortical or medullary parts of the adrenal gland. An inhibited pituitary secretion, with hypoglycemia, is seen in Simmond's disease. The adrenal cortex is affected in Addison's disease with similar effect upon the glycemia. In regard to the thyroid, there is increased susceptibility to insulin after



surgical removal of that gland. In those pathological conditions of the liver in which there is a great liver damage, hypoglycemia is often discovered. Acute yellow atrophy and hepatitis are examples. These may be referred to the inability of the liver to take its usual part in carbohydrate transformations.

### INFLUENCE OF OTHER ENDOCRINE GLANDS UPON CARBOHYDRATE METABOLISM

That the pancreas is not the only gland having an influence upon carbohydrate metabolism has previously been mentioned. This might have been suspected from one curious fact which has been known for a long time. In experimental pancreatic diabetes almost the entire pancreas must be removed before diabetes ensues. Sometimes about one-seventh may be left in connection with the pancreatic duct, to provide the digestive fluid, but that is the maximum. Yet many human diabetic cases that come to autopsy have apparently no destruction of pancreatic tissue whatever. It would seem that if the pancreas alone were involved, more degenerative changes would be seen by the pathologist. It is known that some clinical disturbances of the adrenal and pituitary glands are associated with hypo- or hyperglycemia. The effect of stimulation of the adrenal medulla and of injection of adrenaline has been mentioned before. This internal secretion has some control over both liver and muscle glycogenolysis. Moderate dosage will first lower liver glycogen and muscle glycogen and then raise liver glycogen, because muscle glycogen is changed to lactic acid which is carried to the liver and enters into liver glycogenesis. A higher dosage of adrenaline will result in a decrease of both muscle and liver glycogen because of the loss of sugar in the urine.

Acromegaly is a hyperpituitary condition. It is often accompanied by hyperglycemia and glucosuria. It is an affection of the anterior pituitary gland (see Chapter 23 for fuller discussion of the endocrine glands). Thus it appears that the anterior pituitary secretion is antagonistic to that of the islands of Langerhans. This is not an antagonism of a chemical nature. That is, they do not neutralize each other *in vitro*, but they have opposing physiological effects. An anterior pituitary extract will accordingly cause hyperglycemia upon injection and will oppose the effect of a simultaneous injection of insulin. Removal of the anterior pituitary gland (hypophysectomy) renders the animal hypersensitive to insulin. Houssay showed that if a hypophysectomized animal were later depancreatized, either no diabetes resulted or only a very mild form. Both the diabetes preventive substance (insulin) and its antagonist formed by the pituitary are absent in such an animal. An animal having this double operation is known as the "Houssay animal." The active substance of the anterior pituitary may be termed the insulin-antagonizing factor, or pancreaticotropic substance. Its influence on hexokinase and the opposing action of insulin were mentioned on page 440.

A second influence of this gland was discovered by Young and is called the "diabetogenic" action. If anterior pituitary extract is injected daily in large amount into dogs, a permanent state of diabetes results in from fifteen to thirty days. This seems to be due to a destruction of the  $\beta$  cells and complete

of insulin from the pancreas. This is similar to the action of alloxan. (Ham and Haist.) Both influences of the anterior pituitary are now considered to be due to the action of the growth hormone. (See page 623.)

In Addison's disease, a pathological condition of the adrenal cortex, the blood sugar is usually very low. This can be imitated experimentally. Removal of both adrenal glands has the same effect, and it has been demonstrated to be due to the loss of the cortex rather than to the medulla. Furthermore, although adrenal cortical extract by itself has no influence, it can intensify the inhibitory effect of anterior pituitary extract upon the glucokinase phosphorylating reaction. (Price, 1946.) This also demonstrates the antagonistic relationship between the adrenal cortex function and that of the internal function of the pancreas. Long and Lukens showed a relationship similar to Houssay's for the adrenal and pancreas. That is to say, adrenalectomy attenuates the diabetes resulting from depancreatization. In such "Long" animals, administration of the hormone of the adrenal cortex accentuates the diabetic condition.

The thyroid, too, has an influence upon carbohydrate metabolism. Hyperthyroidism, as exemplified by exophthalmic goiter, is often accompanied by slight hyperglycemia and glycosuria. At first glance this is paradoxical since increased thyroid activity is associated with heightened metabolism, which would seem to require a greater utilization of blood sugar. However, the explanation seems to lie in an increased rate of hepatic gluconeogenesis. The thyroid hormone also aids in absorption of glucose from the intestinal canal. In any rate, hyperthyroidism is usually accompanied by hyperglycemia and hypothyroidism by hypoglycemia.

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## Chapter 17

### LIPID METABOLISM

Although lipid metabolism embraces the metabolism of all types of lipids, most attention will be devoted to the fats. The major part of the lipids of the diet is made up of fats, and our knowledge of the biochemistry and physiology of these compounds is greater than that concerning other lipids. Much of our basic knowledge of the metabolism and transport of the fats we owe to the studies of Bloor and his students. Recently, however, there has been a tremendous increase in experimental work on all lipids, not only the fats, but also the phospholipids and sterols.

#### PHYSIOLOGICAL VALUE OF FATS

Since the lipids are in general poor conductors of heat, their presence in the subcutaneous tissues tends to prevent loss of heat from the body. The greater the amount of fat, the more effective is this heat insulation. This is one very good reason why persons who have an exceptionally thick layer of fat are more comfortable in winter and less comfortable in summer than thinner folk.

Chemically, fats are the best heat producers of the three chief classes of foodstuffs. Carbohydrates and proteins each yield about four large calories of heat for every gram oxidized in the body, while fats yield nine large calories—more than twice as much. This is because there is relatively more carbon and hydrogen in relation to oxygen in fats than in proteins or carbohydrates. In other words, the fats are compounds which are less completely oxidized to begin with and therefore can be oxidized further and yield more heat. For example:

	C	H	O
Glucose, $C_6H_{12}O_6$ , contains	40%	7%	53%
Proteins (approx.)	50	7	25
Fats, e.g., $C_{57}H_{110}O_6$ (tristearin)	77	12	11

#### ABSORPTION OF FAT

There are two hypotheses for the digestion and absorption of fat, the Lipolytic Hypothesis, which has been generally accepted in the past, and the more recent Partition Hypothesis.

**The Lipolytic Hypothesis.**—The neutral fats of the food, triglycerides of the various fatty acids, are emulsified and digested to their constituent fatty acids and glycerol. This digestion, according to this hypothesis, is complete, or nearly so, and occurs chiefly in the small intestine, gastric juice having little lipolytic activity. Animal and vegetable fats seem to be equally well digested:

and absorbed but vary with their melting points. Those melting below 50° C. are practically completely digested, while those melting higher are less well digested. This is probably related to the degree of emulsification. Absorption of the lower melting fats is also better. (Crockett and Deuel.) No absorption of fat or its products has ever been shown to occur in the stomach. It occurs entirely in the small intestine. In the duodenum the presence of bile aids both the digestion and absorption of fats in various ways. It activates the pancreatic lipase and provides a favorable hydrogen ion concentration for its activity. The bile salts lower surface tension and thus promote emulsification, to offer a greater surface for enzyme action. The bile salts also aid in the absorption of fatty acids by forming water-soluble complexes. At least 70 per cent of the fat absorbed goes by way of the lacteals to the thoracic duct and thence into the blood stream. The remainder presumably is carried by the portal circulation. Whether any emulsified, undigested fat is absorbed or not is a question, but since digestion of the fat to fatty acids and glycerol is assumed to be almost complete, there would seem to be little undigested fat available for absorption in that form.

In the course of absorption through the intestinal mucosa, the fatty acid and glycerol are resynthesized to fat. This is not a simple matter. In the mucosa there is a phospholipid which acts as an intermediary between the fatty acids in the intestine and the fats sent into the lacteals. As fatty acid is absorbed into the intestinal cell it reacts with the phospholipid to form fat and the phosphoric acid-choline complex. This complex takes on more glycerol and fatty acid as they are absorbed to regenerate a phospholipid. This explanation is based on the fact that the total phospholipid content of the intestinal mucosa does not change appreciably during fat absorption, but the type of fatty acids found corresponds with those fed. After resynthesis the fat is extruded from the cell into the lacteals. A comparatively small amount goes by way of the portal vein to the liver.

**The Partition Hypothesis.**—According to Frazer and his colleagues, pancreatic lipase does not digest fats completely, either in vitro or in vivo. No glycerol can be isolated from such digestions. The triglycerides are digested to di- and monoglycerides and fatty acids. It also appears that a mixture of the lower glycerides with bile salts and fatty acids furnishes the best medium for emulsification of fats. Consequently, after an initial digestion of a small amount of fat to mono- or diglyceride and fatty acid, these compounds, together with bile salts, start to emulsify the rest of the fat. This may occur with a minimum of agitation. The emulsified fat may now be more easily digested because of the vast surface area available, but a great deal of it remains as emulsified triglyceride. The initial stages of lipolysis may be rapid, but the rate soon lessens, before 30 per cent of the contained fatty acids have been liberated. This is largely because it is only the initial flow of pancreatic juice that is high in lipase.

In regard to absorption, the fatty acids are absorbed to some extent as bile salt-fatty acid complexes, but this is not as extensive as was formerly believed to be the case. There is not enough bile available. However, according to the



Partition Theory there is not as much fatty acid liberated as would be expected if the fats were completely hydrolyzed. They are absorbed via the portal vein and pass to the liver. The glycerides, including the fat globules, are absorbed directly through tiny canals in the membranes of the intestinal mucosal cells and go by way of the lacteals, thoracic duct, and systemic circulation to the fat depots. To repeat, the fatty acid fraction goes to the liver and the glyceride to the fat depots. Recently some doubt has been cast upon the validity of this differentiation of pathways of absorption, at least as regards the rat. Fatty acids, labeled in various ways, have been recovered from the lymph in the same amounts whether given as free acids or as glycerides. (Bloom; Reiser and Bryson.) Apparently some of the ingested labeled fat was hydrolyzed completely, and the glycerol liberated was not utilized for resynthesis of fat but followed an independent metabolic pathway. However, there was evidence of the formation of a considerable amount of monoglycerides. (Reiser, 1952.)

### FAT FROM CARBOHYDRATES AND PROTEINS

In addition to the fat derived from food fat, it is well known that body fat may come from carbohydrate and protein. This has been discussed under carbohydrate metabolism (Chapter 16) since it involves a change in the carbohydrate molecule. The non-nitrogenous portion of some of the amino acids is converted into glucose, and this glucose, as well as other utilizable sugars, is changed to fat. The proof that carbohydrates are changed to fatty acids has been furnished by Schoenheimer and Rittenberg. To the drinking water of mice was added heavy water so that the concentration of " $D_2O$ " in all of the body fluids became constant. The animals were on a high carbohydrate diet. The deuterium content of the fatty acid rose rapidly. Since all synthetic reactions involving tissue fluids were using a proportionate amount of heavy water, this result indicated that carbohydrate and water were entering into the synthesis of fatty acid.

Positive demonstration of the synthesis of fat from protein has been given by Longenecker. He fed a diet containing casein, salts, and yeast, i.e., almost no carbohydrate or lipid, to rats which had been fasted to deplete them of their stores of fat. They regained their original weight on this diet and analysis of the tissues showed that a large percentage of the new tissue was fat.

Chaikoff fed glucose labeled with  $C^{14}$  to mice. The isotope was subsequently found in tissue palmitic acid. This occurred largely in the liver, but also in other organs. On a high carbohydrate diet this fatty acid formation exceeded glycogenesis.

For the synthesis of fat from carbohydrates, thiamine is needed, and in the synthesis of fat from protein, pyridoxine is also required, as well as thiamine and perhaps other members of the vitamin B complex. (McHenry and Gavin.)

### TRANSPORT OF FAT

Since both fat and fatty acids are insoluble in water, consideration must be given to the means whereby they are carried in the blood and lymph. Some of

fatty acid, perhaps in the bile salt-fatty acid combination, is carried in the portal circulation as far as the liver, where the bile salt is detached and secreted into the bile. Most of the fat, however, is resynthesized probably in the way described. Some of the fat globules are incorporated into leucocytes, which carry them to the lacteals. But just how most of the resynthesized fat passes out of the intestinal epithelial cells and into the lacteals is not known. The lacteals carry their contents into the celiac lymph nodes and into the superior mesenteric lymph nodes, which empty mainly into the intestinal lymph trunk. Thence the fat is into the thoracic duct, through the cisterna chyli, and finally into the blood system at the junction of the left internal jugular and subclavian veins. The fat, then, minutely subdivided, may be seen as microscopic particles, "chylomicrons," in the blood. If a sample of blood is taken four or five hours after a meal rich in fat, the plasma will be seen to be quite turbid or even milky, due to the presence of large amounts of suspended fat.

All of the fatty acid present in blood is not in the form of neutral fat. Cholesterol carries some fatty acid as cholesterol esters. The proportion of cholesterol as esters is normally about 60 to 70 per cent of the total cholesterol in the plasma. The phospholipids are not important in fatty acid transport in the blood, although, as mentioned before, they function in the intestinal mucosa during absorption. When palmitic acid, tagged with  $C^{14}$  in the carboxyl group, was fed to rats, 96 per cent of the tagged acid was recovered in forms other than phospholipids. (Bloom, 1951.)

In view of the fact that blood plasma cannot be completely freed of lipids by extraction with organic solvents, it has long been evident that they do not exist in the free state. A considerable proportion is combined with protein. These appear to be in the globulin fraction. The " $\beta_1$ -lipoprotein" was found to contain 8 per cent free cholesterol, 39 per cent cholesterol esters, and 29 per cent phospholipids. Most of the plasma cholesterol is present in this form. (Necley.)

### Changes Occurring in the Liver

A large proportion of the absorbed fat is carried to the liver for temporary storage. This includes not only any fat which may go by way of the portal circulation, but also the fat circulating in the systemic circulation. Here a

TABLE XXXIV  
PLASMA LIPIDS OF NORMAL YOUNG WOMEN\*

	MG. PER CENT
Total lipid	589
Neutral fat	154
Total fatty acid (iodine number, 88.5)	353
Phospholipid fatty acid (iodine number, 124)	130
Cholesterol ester fatty acid	77
Neutral fat fatty acid	146
Total cholesterol	162
Combined cholesterol	115
Free cholesterol	47
Phospholipid	196

\*After Boyd, E. M.: J. Biol. Chem. **101**: 323, 1933.

very large part, if not all, is transformed to phospholipid, in which form it may be sent into the blood stream for distribution to the diverse organs and tissues. If this transformation to phospholipid is prevented, there is a deposition of fat in the liver, that is, "fatty liver" occurs, which is an abnormal and serious condition. For the building of lecithin, choline of course is needed, or the precursors from which choline can be produced. Therefore, choline or its precursors will prevent, or cure, fatty livers. From Table XXXIV it will be seen that the phospholipids are present in blood plasma in greater amount than either cholesterol or neutral fat. The liver plays other important roles in fat metabolism which will appear later on.

### FATE OF FAT IN THE BODY

Fats and other lipids are (1) oxidized to provide heat and energy, (2) stored for future utilization, (3) secreted in milk, or (4) excreted in the feces. Much of the milk fat originates in the neutral fat of blood, which in turn comes most readily from food fat. Feeding large quantities of cottonseed oil to cows will change the properties of the butterfat considerably. Although fat can be derived from carbohydrate and from protein, a lactating animal cannot produce milk of high fat content if the diet is too low in fat. That is, synthesis cannot quite keep pace with the demand for fat. The utilization of intravenous administered fat has been discussed on page 406.

The total lipids of the feces make up about one-third of their dry weight. There is very little neutral fat or phospholipid present in this. In fact, most of the fecal lipids are not food residues which have escaped digestion and absorption. The distribution of the fatty acids in the fecal lipids more nearly resembles that of the blood than of the food. This suggests that they represent a secretory product. Moreover, they continue to be found in the stools even during fasting as well as when a bile fistula is present. It thus appears that normally the fecal lipids are not chiefly derived from bile but that the intestinal mucosa excretes most of the fatty acids found in the feces. It also excretes some cholesterol. The plant sterols of the food, as well as excess cholesterol of the food, go right through the intestinal canal into the feces. Bacteria change some of the cholesterol to coprosterol in the intestine and possibly also effect changes in plant sterols.

### Storage of Fats

The fats and other lipids are deposited in various tissues of the body. If an animal is starved for a long time, there will still be found lipid in the tissues. This is not fat but probably phospholipid. It has been called the *élément constant* and is independent of previous feeding. It is believed that this lipid is essential to the life of the cells. This is structural or functional material essential to the framework of the cell or to its proper activities. Lipids which are stored in excess of this have been termed *élément variable*, which indicates that the amount is quite inconstant. This represents the excess of intake over immediate utilization and when deposited in large masses is called *depot fat*.



which case it is the true adipose tissue. It is stored primarily for its fuel value but secondarily has other uses. Thus, it is an insulator against heat loss; it serves to pad joints, nerves, and organs against shock; it may support kidneys or other organs. In visceroptosis the abdominal viscera drop because of little support. This may occur when rapid loss of weight in obese individuals moves the supporting adipose tissue. Other functions include transformation to milk fat or into other lipids, such as the phospholipids, sterol esters, or glycosides, or to be used as fat for the fetus.

Each species of animal tends to store a characteristic mixture of fats. Merely to mention lard, mutton fat, chicken fat, and fish oils will indicate how it is. And yet this characteristic fat can be modified by feeding large enough quantities of some unusual lipid. Unsaturated fats, low molecular weight fats synthesized by the animal, and halogenated fatty acids are examples of lipids which have been found to modify the body fats of animals to which they are fed. Feeding mutton fat with a high melting point to a dog raised the melting point of the animal's fat from about 20° to about 40° C., while linseed lowered it to 0° C. (Lebedeff). This is unusual and is due to forcing the "foreign" fatty acid. Under normal conditions the animal deposits fat having a melting point not far from its own body temperature. It is therefore evident that in order to deposit its own peculiar fat under ordinary circumstances the organism must oxidize unwanted fatty acids, retain those which are suitable, use others, and even synthesize some.

Until quite recently it was the general opinion that the body used or oxidized as much fat as it needed, stored the rest, and that these fat deposits remained, like hoarded gold, inactive until withdrawn for use. This is not the case. The experiments of Schoenheimer and his co-workers, and of Eckstein, Engenecker, and others are responsible for this change in scientific opinion. Using fats containing fatty acids tagged with deuterium, the following facts have been brought out. Even small amounts of tagged fatty acids in the diet are first incorporated into body fat before they are oxidized. Since no changes in body weight or in total body fat occurred in these experiments, it is evident that there must have been a simultaneous removal of fatty acids from the body to make room for the dietary fat. This is further shown by the fact that the deuterium rapidly disappears from the body fat as soon as the dietary fat is changed to untagged fat. Apparently before fatty acid can be oxidized it must be incorporated into the body fats. The body fat, therefore, is not an inert mass but is a part of a dynamic system, the fatty acids being continually deposited and withdrawn. This deposition of marked fatty acids only occurs when they are of rather high molecular weight (above  $C_{10}$ ). Those of low molecular weight are directly consumed for energy.

**Fatty Livers.**—Excessive amounts of fat in the liver have been seen by pathologists at autopsy for many years. Fatty "degeneration" and fatty infiltration are terms for some of these conditions. Consequently the experimental study of fatty livers must be of more than passing interest. The fatty "degeneration" is a physical change in the cell and does not neces-

sarily involve a change in the amount of fat. In fatty "infiltration" there is an increase in the fat intracellularly. Most of the "fatty livers" to be discussed under this heading are instances of fatty "infiltration."

Depancreatized dogs, besides becoming diabetic, develop fatty livers. This occurs even if the animal is treated with insulin to control the hyperglycemia. Feeding raw pancreas can prevent the condition from occurring. The explanation first offered was that the raw pancreas furnished the pancreatic enzymes which were absent in a depancreatized animal. Later it was shown that lecithin had just as good an effect as pancreas, and finally it was narrowed down to one component of lecithin, namely, choline (Hershey). Other substances which affected this type of fatty liver were betaine and triethylcholine. Such substances are called "lipotropic," which simply means that they prevent, or cure, fatty livers. Certain proteins, notably casein, are also lipotropic. This was shown by Eckstein to be related to their high content of methionine, and a combination of two or more amino acids, in addition to methionine, is needed for a complete lipotropic action. (Elvehjem.) Cystine, however, if fed together with a low choline diet, tends to cause fatty livers, and there are a number of other methods of inducing this condition.

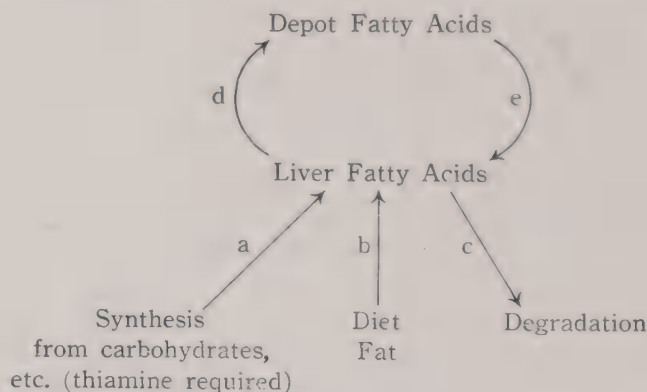


Fig. 56.—The sources and fates of liver fatty acids. (After Stetten and Salcedo.)

In attempting to account for some of these phenomena, the diagram in Fig. 56 may be of some assistance. This indicates the sources and fates of liver fatty acids and their relationship to depot fatty acids. Part of the explanation lies in the fact that a great deal of the fatty acid is converted into phospholipids, and choline is required for their formation. These, as has been stated before, must be formed from fats in the liver before they can be sent forth to be used by the tissues. If choline, or the "makings" of choline are not present, the fats will accumulate and clog up the liver. It will be remembered that the animal organism cannot readily synthesize methyl groups. These are mostly obtained by "transmethylation"; that is, the transfer of labile  $\text{CH}_3$  from appropriate donor compounds to acceptors. Methionine, betaine, etc., are lipotropic because they can transfer their methyl groups to ensure the synthesis of choline. Then choline unites with phosphoric acid, fatty acids, and glycerol to form lecithin in the liver and replace the neutral fat with this phospholipid.

low choline diet, therefore, will produce fatty livers because of impaired transportation of fatty acids from liver to the fat depots via route d. Cystine has an opposite effect to choline because it accepts methyl groups.

This seems a simple and logical explanation. It is not, however, the whole story. In treating a depancreatized dog with choline, at least 1 Gm. a day is needed. The effective amount of raw pancreas supplies only about 6 per cent of this requirement. Dragstedt and his associates prepared an extract of the pancreas which they considered an internal secretion (in addition to insulin) and which they named "lipocaic." This was effective in preventing or curing fatty liver. There is a great deal of controversy regarding lipocaic, some supporting Dragstedt's contention, others stating that it owes its efficacy entirely to choline, and still others maintaining that pancreatic juice is just as effective as the pancreatic tissue extract. McHenry suggests that the fatty liver of the depancreatized dog is due to the positive action of a toxic substance present in the meat of the diet, together with a failure of digestion which prevents the liberation of a lipotropic factor. The "toxic" substance may be biotin, which under ordinary conditions has a beneficial physiological effect. Pancreatic extracts contain the free lipotropic factor which is absorbed and tends to prevent fat deposition. This factor may possibly be inositol. The diet usually contains choline and protein, with its methionine, which are also lipotropic. If raw pancreas is fed, it aids in the digestion and liberation of the lipotropic factor, methionine.

Fatty livers may also be induced by feeding an excess of cholesterol or an excess of fat. This would appear to be an increased rate of fatty acid formation along route b. In both cases choline will only diminish the deposition of that fraction of lipid consisting of neutral fats. It has little or no effect upon the cholesterol fraction. Inositol, another lipotropic agent, has a marked effect in reducing the amount of cholesterol in the liver. Choline also has no inhibiting action on the fatty livers produced by phosphorus poisoning or certain other specific hepatic poisons. It is interesting to note that diets which produce fatty livers fail to do so unless thiamine is present. That is, diets low in choline, whether high or low in fat, will not result in fatty livers in the absence of vitamin B<sub>1</sub>. The vitamin is probably essential for the conversion of carbohydrate to fat, and route a indicates this line of action. Riboflavin has a similar effect. The feeding of cystine also seems to result in increased synthesis (route c), as well as in antagonism to choline.

When anterior pituitary substance or the "ketogenic" fractions of anterior pituitary extract are administered to animals, fatty livers result. This is caused by excessive mobilization of depot fat and its migration to the liver. This is a stimulation of process e and cannot be prevented by choline. (Stetten.)

To summarize:

1. Fatty livers caused by depancreatization are prevented by (a) large amounts of choline or labile methyl donors or (b) by lipocaic. The labile methyl donors produce choline, which is needed for lecithin formation. Lipocaic



may owe its action to a vitamin, inositol. Raw pancreas is also effective because it aids in the digestion of food and liberation of inositol and methionine. Moreover, plant proteases, when fed, also prevent fatty liver formation. (Feinberg.)

2. The same treatment is effective in fatty livers, resulting from a low choline diet, high fat feeding, and with respect to the neutral fats deposited, from high cholesterol diets.

3. Choline or lecithin feeding does not affect fatty livers, which are due to phosphorus poisoning or to anterior pituitary administration, since in both cases the accumulation of liver fat is derived from the fat depots of the body.

**Obesity.**—Obesity is “that state in which the accumulation of reserve fat becomes so extreme that the functions of the organism are interfered with.” It may arise from (a) overeating or (b) diminished utilization, or a combination of both. Various theories have been proposed to explain how this occurs. Among these is the hypothesis that the basal metabolic rate is low and, therefore, with a normal intake of food an excess of calories is available. As will be seen, the basal metabolic rate diminishes slightly as the individual grows older. Often his food consumption is not decreased proportionately, and as a consequence obesity may result. Otherwise obesity cannot be accounted for by a diminution in the basal metabolic rate. In fact, the heat production of obese individuals is usually above normal, except when the obesity is associated with hypothyroidism. It has also been claimed that obese individuals show lower specific dynamic effects of foods than do normal people (see Chapter 21). This, too, has been proved incorrect. Nevertheless, persons afflicted with extreme obesity frequently cannot lose weight in spite of most rigorous undernutrition. Since the laws of conservation of matter and energy operate under all circumstances, it is possible that the answer to this problem may be found in a study of the water balance (see page 569). The person’s tissues retain water more tenaciously than normally and, perhaps, water takes the place of the fat which is lost. Just how the endocrine glands fit into the picture is difficult to explain. They may control water balance; they may influence the patient’s “urge” for work. It is fairly definitely established that they do not directly cause the formation of adipose tissue, although they do seem to influence the pattern of the distribution of such tissue.

## OXIDATION OF FATTY ACIDS

It is usually assumed that the glycerol fraction of the fat is handled in much the same way as the carbohydrates. We have seen that three carbon chains arise from a splitting of the hexoses and are oxidized eventually to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , and there is no reason why glycerol from fat should not follow a similar path. Probably there is first a phosphorylation and an oxidation to 3-phosphoglyceraldehyde or to some similar derivative. The path shown on page 400 will show the possible further route. That glycerol can enter into synthetic carbohydrate reactions is seen in the fact that, when glycerol is fed to diabetic animals, it is excreted as glucose.

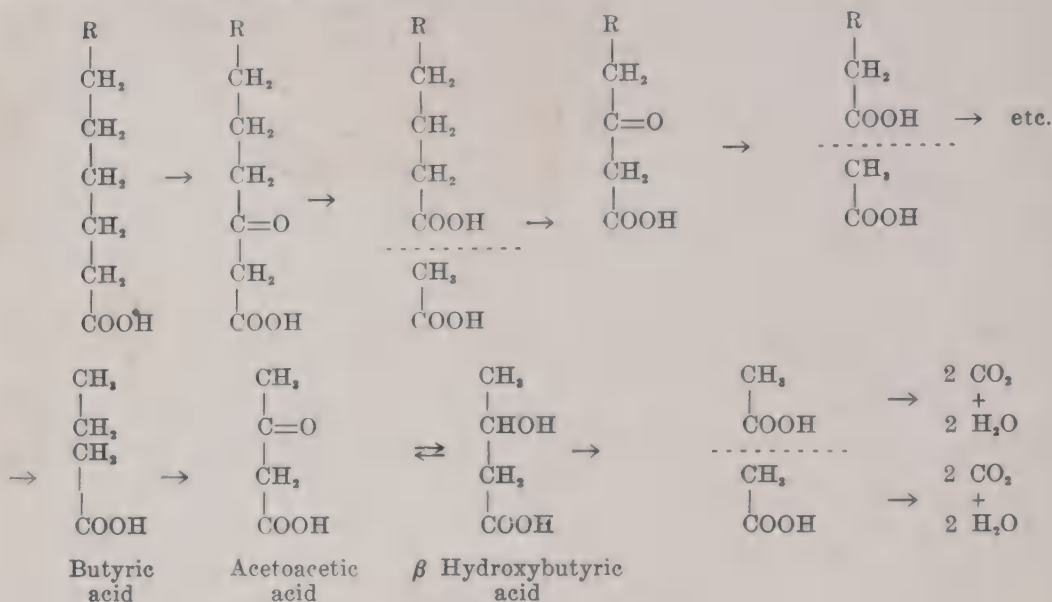
The oxidation of the long-chain fatty acids has been the subject of many investigations. Leathes formulated the hypothesis that the first step in the breakdown of a long-chain fatty acid was its desaturation in the liver. The evidence for this lay in the fact that the liver fatty acids are more unsaturated than those present in other tissues. Direct proof that desaturation occurs (although not necessarily in the liver) has come from isotope experiments. Saturated fatty acids containing deuterium when fed have been isolated as unsaturated acids. Saturation of unsaturated acids also occurs, as well as the lengthening or shortening of the chains (Schoenheimer). Apparently desaturation occurs only in definite patterns and not in others. Thus the "essential fatty acids," linoleic and linolenic acids, cannot be formed in the animal body. They are the homologs of oleic acid, with two and three double bonds, respectively, instead of one. At the present time it is not definitely known whether or not desaturation is a prerequisite for oxidation of fatty acids. However, it undoubtedly does occur, but to the extent of only one double bond in each molecule. Since only one double bond can be introduced into a fatty acid by the body, it is evident why linoleic and linolenic acids must be ingested and are, therefore, "essential." Chemically a double bond is a definite point of weakness and would seem to render the chain more susceptible to oxidation. The introduction of a double bond is catalyzed by a dehydrogenase of the liver.

The most generally accepted hypothesis of fatty acid oxidation is the one called beta-oxidation. When fatty acids of different lengths are fed to man or animals, no derivatives can be isolated from blood or urine which will throw any light on the mechanism whereby they are broken down. Consequently Knoop conceived the idea of tagging the fatty acids in 1904, a time when isotope experiments were not possible. His method was to feed the phenyl derivatives and isolate the compound containing the benzene ring from the urine. Benzoic acid is not oxidized by the body but combines with glycine to form hippuric acid,  $C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$ . The next higher acid, phenyl acetic acid, was eliminated as phenaceturic acid,  $C_6H_5 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$ , a combination of phenylacetic acid with the same amino acid, glycine. Now, on feeding the third in the series, phenylpropionic acid, the next higher homolog to phenaceturic acid was not formed, but hippuric acid instead. Going up one step further, phenylbutyric acid was transformed to phenaceturic acid. That is:

$C_6H_5 \cdot COOH$	eliminated as	$C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$
$C_6H_5 \cdot CH_2 \cdot COOH$	eliminated as	$C_6H_5 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$
$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot COOH$	eliminated as	$C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$
$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$	eliminated as	$C_6H_5 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$

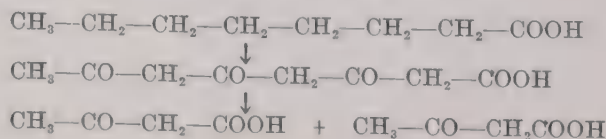
Inspection of this series will show that the first and third set have the same decomposition product. In order to accomplish this, phenylpropionic acid had to lose two carbons. The same relationship exists between the second and fourth set. The conclusion was reached that the fatty acid chain loses two carbon atoms at a time, i.e., that oxidation starts at the beta carbon and when this results in the loss of two carbons, the new beta carbon is attacked, and so on.

With a long-chain fatty acid, we would have, on the basis of this hypothesis, a chain of reactions somewhat as follows:



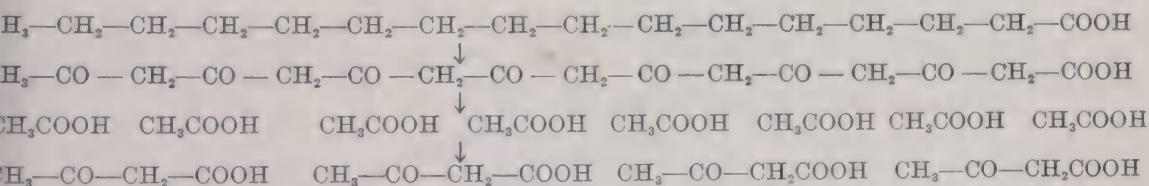
There is a good deal of additional evidence that beta oxidation is at least the first step in fatty acid oxidation. Under most circumstances, four-carbon chains seem to result from fatty acid degradation. Perfusion of isolated livers with blood containing fatty acids results in the formation of acetoacetic acid (Embden). Similar results have been obtained by Dakin by the oxidation of fatty acids *in vitro*. More recently, Schoenheimer and Rittenberg have shown that tagged stearic acid (18 C's) when fed could be isolated as palmitic acid (16 C's). The reverse also occurred.

It will be noted that in beta oxidation,  $\beta$ -hydroxybutyric acid and acetoacetic acid are *normal* intermediate products. As will be seen later, they appear in blood and urine in large amounts under certain abnormal conditions. It will be evident that it is not their production which is abnormal but rather their overproduction; i.e., the formation of an excess of these four-carbon chains beyond the capacity of the extrahepatic tissues to utilize them. In beta oxidation, as outlined, only one molecule of acetoacetic acid should be produced from each molecule of fatty acid. When certain fatty acids are fed under suitable conditions, more than one molecule of acetoacetic acid can be recovered from the urine (Deuel). Probably palmitic, stearic, and oleic acids break up into at least three fragments per molecule, each of which is capable of transformation into diacetic acid. Each fragment then undergoes beta oxidation. It is also probable that caproic and butyric acids are broken down by beta oxidation. The eight- to fourteen-carbon chains may undergo "multiple alternate oxidation." (Hurtley.) This may be illustrated by the following scheme for octanoic acid.

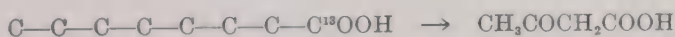




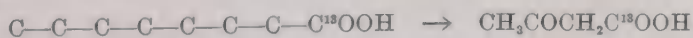
A more recent theory is that of Mackay and his co-workers. It may be called the "beta oxidation—condensation" theory. It is based on studies *in vitro* of the effect of liver tissue upon fatty acids (Jowett and Quastel), and upon feeding experiments. Propionic acid ( $C_3$ ) did not yield ketone bodies but did lead to the production of glycogen. Butyric acid ( $C_4$ ) produced ketones; i.e., it was ketogenic, as were also valeric ( $C_5$ ) and hexanoic ( $C_6$ ) acids. The  $C_6$  compound yielded more ketone bodies than the  $C_4$  compound. This fact could not be explained by the orthodox beta oxidation theory nor by multiple alternate oxidation. Furthermore, the  $C_5$  acid gave rise to glycogen and ketone bodies, and the  $C_7$  acid produced glycogen and more ketone bodies. This seemed to indicate that a  $C_2$  chain might be converted into ketone bodies, and, indeed it was found that when acetic acid was fed to fasting rats or phlorizinized dogs, acetone bodies were excreted in the urine. The theory is that all fatty acid chains, odd or even, are oxidized at each alternate carbon atom, as in "multiple alternate oxidation." The molecule then splits at every keto group to form acetic acid molecules, except where a three-carbon chain is left. Such a chain forms propionic acid which is glycogenic, not ketogenic. (However, this would not happen in the case of naturally occurring fatty acids because all of them have an even number of carbons in their chains.) Pairs of acetic acid molecules then condense to form acetoacetic acid. The following scheme will illustrate further:



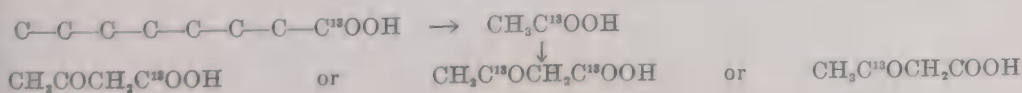
An ingenious experiment has recently been described which supports the beta oxidation condensation theory. Rat liver slices were incubated with octanoic acid, containing isotopic carbon ( $C^{13}$ ) in its carboxyl group. If oxidation of this acid occurred by simple beta oxidation, there would result acetoacetic acid containing no  $C^{13}$ , because the carboxyl end is oxidized off, two carbons at a time.



In oxidation by multiple alternate oxidation, the  $C^{13}$  should be only in the carboxyl group, which is presumably always split off as acetoacetic acid.



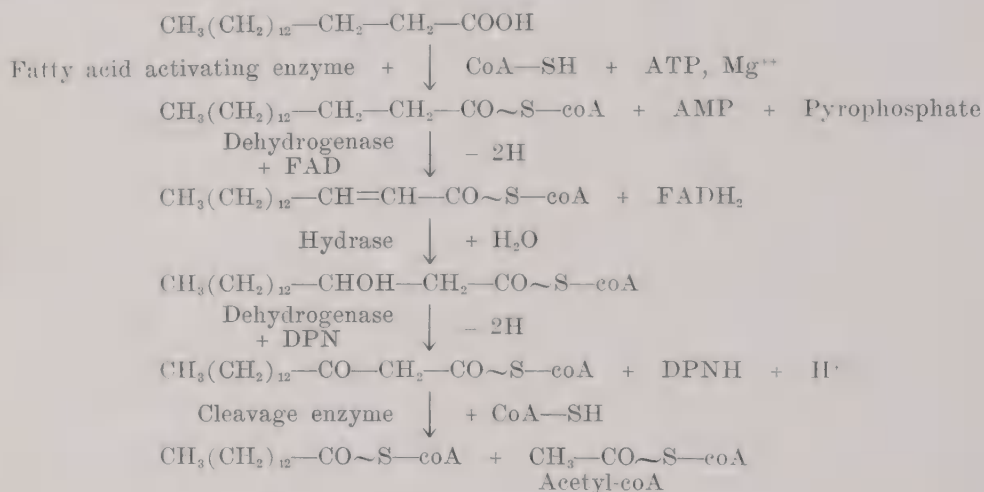
But by beta oxidation-condensation, the  $C^{13}$  should be evenly distributed between carbonyl and carboxyl carbons of the resulting acetoacetic acid. This follows because acetic acid molecules arise first, and some of these supply the carboxyl end and others the ketone end of the acetoacetic acid formed.



The results of the experiments showed some of the  $C^{13}$  in the carbonyl group, indicating that oxidation of fatty acids proceeds in large part by this beta oxidation condensation mechanism. (Weinhouse.) The conclusion to be drawn from these experiments is that at least part of the fatty acids is broken down to two carbon units and then rebuilt to the four-carbon acetoacetic acid.

Further experimentation with isotope-containing compounds indicates that the various organs differ in their ability to metabolize acetate and the ketone bodies. Thus, ketone body formation takes place in rat kidney as well as rat liver, but in the heart muscle acetate is oxidized without the intermediate conversion to ketone bodies. Furthermore, while the liver is unable to break down ketone bodies, the kidney and heart can do so very rapidly.

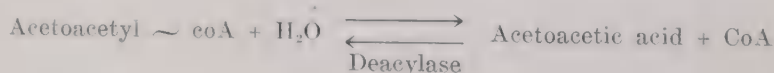
Recently coenzyme A (see page 296) has been shown to be involved in the breakdown and build-up of fatty acid chains, as well as in the utilization of the  $C_2$  fragments in other ways. The work of Lynen, Mahler and others suggests the following scheme.\*



This is, in effect, a beta oxidation, the fatty acid losing two carbons. The process may be repeated, again and again, two carbons being cut off each time. Acetoacetic acid may be formed from two molecules of acetyl-coA, resulting from the above series of reactions, as follows:



In the liver there is an enzyme which hydrolyzes acetoacetyl-coA, liberating acetoacetic acid:



Acetyl-coA, it will be remembered, is the form in which  $C_2$  fragments enter the tricarboxylic acid cycle. (See page 426.) The oxidases concerned in

\*Abbreviations used in this scheme are: ATP, adenosinetriphosphate; AMP, adenosine-5'-phosphate; FAD, flavin adenine dinucleotide; FADH<sub>2</sub>, reduced FAD; DPN, diphosphopyridine nucleotide; DPNH, reduced DPN.

these reactions are firmly bound to the mitochondria of the cells, and they oxidize long fatty acid chains better than short ones.

For the synthesis of fatty acids by the body, two carbon units are used. Short-chain fatty acids, as well as glucose, pyruvate, acetone, and ketogenic amino acids, are degraded into 2-carbon fragments prior to lipogenesis. Gurin has found that extracts of pigeon liver are capable of converting  $C^{14}$ -labeled acetate into long-chain saturated fatty acids. It appears that rapid glycolysis is a primary requirement if this is to be accomplished efficiently, and it is quite likely that synthesis occurs by the reverse of the degradative reactions given above. Long-chain fatty acids may also be lengthened, at the carboxyl end, by the addition of  $C_2$  units. Thus myristic acid ( $C_{14}$ ) was transformed into palmitic acid ( $C_{16}$ ), and palmitic acid into stearic acid ( $C_{18}$ ). These changes also occur in the liver. (Anker; Zabin.) Since coenzyme A levels and the capacity to synthesize lipids run parallel, it is evident that coenzyme A plays an important part in lipid synthesis. This holds for cholesterol as well as for fatty acids. (Klein and Lipmann.)

### KETOGENESIS

The term ketogenesis means the formation of "ketone bodies." The ketone bodies include, besides acetoacetic acid, beta hydroxybutyric acid and acetone. Acetone, however, is merely a breakdown product of either of the other two, which are the really important substances concerned. Attention should therefore be centered entirely on acetoacetic acid (diacetic acid) and beta hydroxybutyric acid. Ketosis is the production of ketone bodies in excess of the ability of the body to utilize them. It occurs in severe diabetes, in starvation, in the "acidosis of childhood," during anesthesia, and it can be precipitated by feeding an unbalanced diet, namely, high fats with low carbohydrate. The appearance of these compounds in the urine is a danger sign, indicating usually an acidosis, and warning the clinician of impending coma. As has been seen, they are normal degradation products of the fatty acids, and it is now accepted, on the basis of work done on liver slices in vitro, that they are formed in the liver. Himwich and his colleagues have corroborated this for the intact animal. The blood flowing from the liver has a higher concentration of ketone bodies than that flowing toward it. Chaikoff and Soskin demonstrated that the removal of the liver from a diabetic dog resulted in a drop in the acetone bodies of the blood. Although other tissues are capable of producing small amounts of these compounds, the liver is by far the chief "ketogenic" organ. It can, in fact, be regarded as practically the only site of ketone production. The formation of ketone bodies is regulated by one of the anterior pituitary hormones, and also, possibly, by a hormone secreted by the alpha cells of the islands of Langerhans of the pancreas. (See pages 627 and 435.)

Under normal conditions the liver breaks down the fatty acids to the ketone bodies and sends them into the blood stream for distribution to the rest of the body. It is in the "extrahepatic" tissues that they are oxidized further; the



liver does not use them to any appreciable extent. This oxidation occurs by way of the tricarboxylic acid cycle. Kidney, muscle, heart, brain, and testes all have been shown to utilize these substances. The end products are  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . It has recently been found that the injection of the products of intermediary fat metabolism can produce pancreatic cellular changes of such a nature that a diabetic condition may result. (Nath and Brahmachari.)

## KETOSIS

Knowing that the ketone bodies are produced by the liver and utilized by the tissues, a ketosis might conceivably be caused by (1) increased production of ketone bodies by the liver, or (2) decreased utilization by the extrahepatic tissues. The most common conditions associated with ketosis are starvation and diabetes. If the tissues of animals suffering from experimental ketosis are tested, they are invariably found to be ketolytic, that is, they utilize the ketone bodies, and are just as active in this respect as tissues of normal animals.

It is, therefore, apparent that in ketosis the liver must produce ketone bodies at a rate exceeding the *normal* capacity of the extrahepatic tissues to burn them. Why should this happen? Let us first study some of the observations made when the ketone bodies were assumed to be abnormal products of fatty acid oxidation. It was known that they occurred in diabetes mellitus, starvation, and in other conditions in which carbohydrate reserves were depleted. Furthermore, if the carbohydrate metabolism could be improved, the acetone production was diminished. This seemed to indicate that the combustion of sugar was necessary for the normal utilization of fats. This conception was fancifully summed up in the statement of Rosenfeld, "Fats burn only in the flame of carbohydrate." In 1921 Shaffer showed that the oxidation of acetoacetic acid *in vitro* is catalyzed by the presence of glucose. He grouped foodstuffs into two classes, ketogenic and antiketogenic, and stated that in order to prevent ketosis there must be a definite ratio between the two classes actually being metabolized in the body. That is, a certain amount of glucose, and other antiketogenic factors must be used in order to oxidize completely the fatty acids and other ketogenic foods. The combustion of glucose was believed to be increased in the diabetic under the influence of insulin and its "antiketogenic" effect could thereby become effective. Dietitians planned diets so that the ketogenic:antiketogenic ratio would be correct. These mathematically planned diets usually were efficacious, but it now appears that their success was a happy coincidence because the hypothesis on which they were based is now generally held to be incorrect. This was proved by Mirsky and his co-workers, when they showed that glucose had no influence on the rate of ketone body utilization by the muscles. It was first demonstrated on eviscerated hepatectomized animals under various conditions and has been corroborated by other types of experiments. The conclusion is that the utilization of the ketone bodies is not affected by the concomitant consumption of glucose or by the presence of insulin. Yet insulin will diminish the ketosis of diabetes, and carbohydrates will do the same for the ketosis of starvation.

It was stated that ketosis must be due to an overproduction of ketone bodies by the liver rather than to a diminished utilization by the extrahepatic tissues. Now it is seen that the consumption of glucose does not cause the body tissues to use more ketone bodies. In fact, they can metabolize only a certain quota of these substances. Stadie has said, "Up to a certain level fat metabolism is complete and there is no ketonuria. Beyond this level fat metabolism is incomplete and part of the fat is excreted in the form of ketone bodies." The reason for the occurrence of ketosis in diabetes is that the body must make up the deficit in carbohydrate calories by burning more fat and more protein. Fat is ketogenic and so are some of the amino acids. The rate of ketogenesis exceeds that of the utilization of the ketone bodies by the extrahepatic tissues, and therefore ketone bodies accumulate in the blood and are excreted in the urine. In starvation the glycogen reserves are depleted first, and then body fat and body protein are called upon for the energy requirement. Again ketogenesis exceeds the ability of the extrahepatic tissues to burn the ketone bodies, and ketosis results. Weinhouse and co-workers suggest that carbohydrate may exert its effect at the 2-carbon level of fatty acid breakdown. Pyruvic acid from carbohydrate may be carboxylated to oxaloacetate which then aids in the metabolism of the 2-carbon derivative formed by  $\beta$ -oxidation of fatty acids, by way of the citric acid cycle.

In diabetes mellitus ketosis can be alleviated by the administration of insulin, usually with carbohydrate, which improves carbohydrate metabolism. The ketosis of childhood is now ascribed to the fact that the young child does not retain glycogen as readily as the adult. Feeding sugar brings about a glycogenesis and relieves the condition.

### Ketogenic and Antiketogenic Substances

The ketogenic substances are, of course, all the fatty acids. In addition, at least three amino acids belong to this group; namely, leucine, phenylalanine, and tyrosine. Antiketogenic substances, in the sense of preventing the formation of the ketone bodies, are the carbohydrates, the glycerol fraction of fat, and the following amino acids: glycine, alanine, serine, valine, cysteine, methionine, aspartic acid, glutamic acid, proline, ornithine, arginine, histidine, and threonine. These are antiketogenic because their non-nitrogenous residues are convertible to glucose.

**Common Pathways of Protein, Carbohydrate, and Fat Metabolism.**—The conversion of protein to carbohydrate and of carbohydrate to fat has been discussed previously. (See page 417.) The conversion of carbohydrate to fat has been demonstrated experimentally. When mice were fed glucose labeled with  $C^{14}$ , palmitic acid subsequently recovered from liver and other organs contained the carbon tag. On a high carbohydrate diet, fatty acid formation may exceed glycogenesis. (Chaikoff.) The question of the transformation of fatty acids to carbohydrates has been a much debated one for many years. However, the question is no longer significant in view of the fact that fatty acids are largely catabolized to a two-carbon stage. As acetyl-coA these may condense with oxaloacetic acid and thus enter the tricarboxylic acid cycle.

Moreover, the conversion of fat to carbohydrate has been directly demonstrated. Palmitic acid-6- $C^{14}$  was injected into a diabetic dog. The urinary glucose contained  $C^{14}$ . (Abraham.)

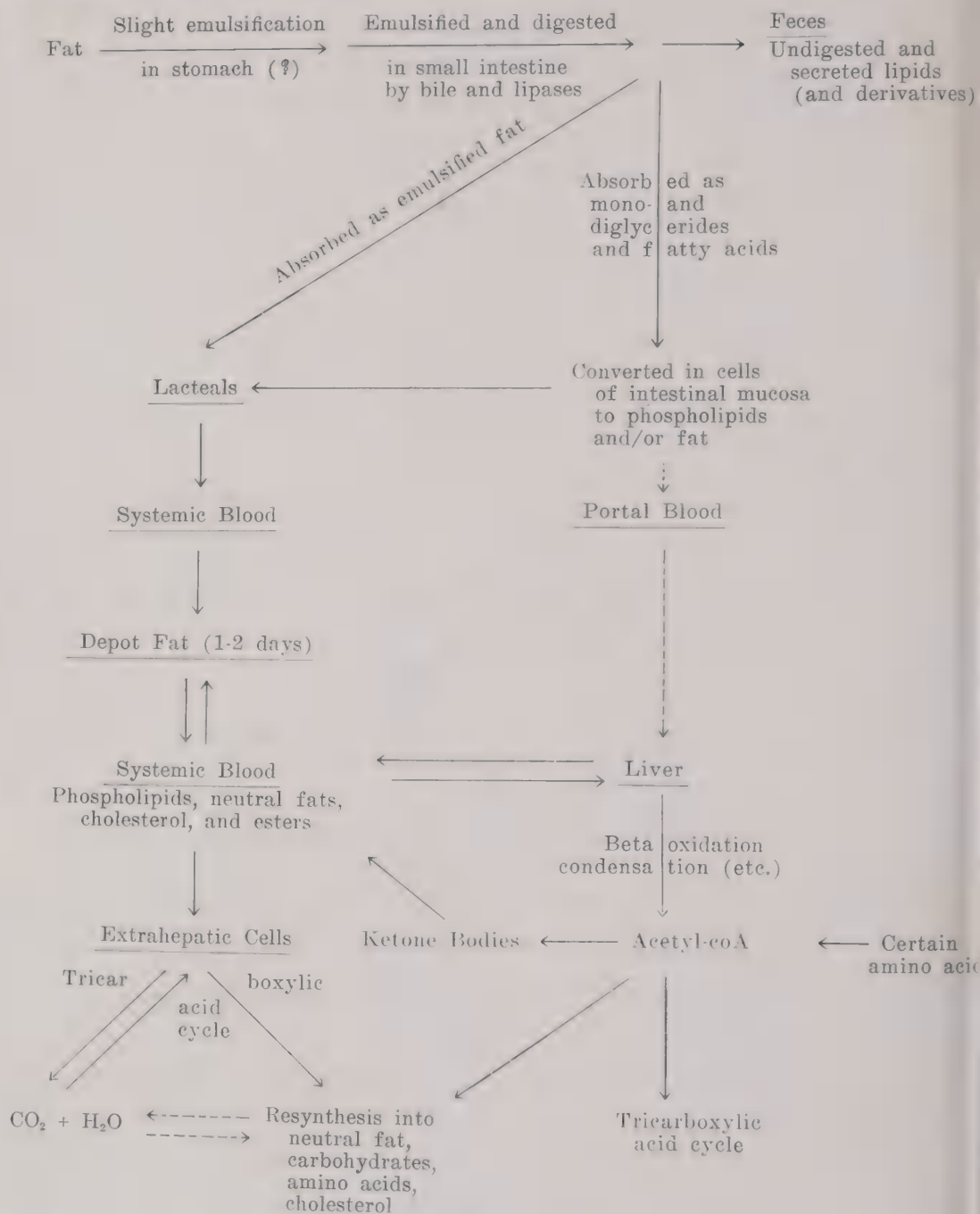


Fig. 57.—Outline of fat metabolism (broken lines indicate possible routes). (Courtesy of Dr. Harry Baron.)

Since many amino acids are glucose formers and others are acetoacetic acid formers, it follows that they also can be metabolized in the same cycle. Glutamic acid may also be converted to  $\alpha$ -ketoglutaric acid and thus enter the cycle at that point. The tricarboxylic acid cycle therefore is a common pathway of metabolism.



bolism for all three classes of foodstuffs and thus the question of interconvertibility becomes of no moment. (See Fig. 58.) In the outline in Fig. 57 are shown the pathways of fat and its metabolites, as well as the relationship of the tricarboxylic cycle.

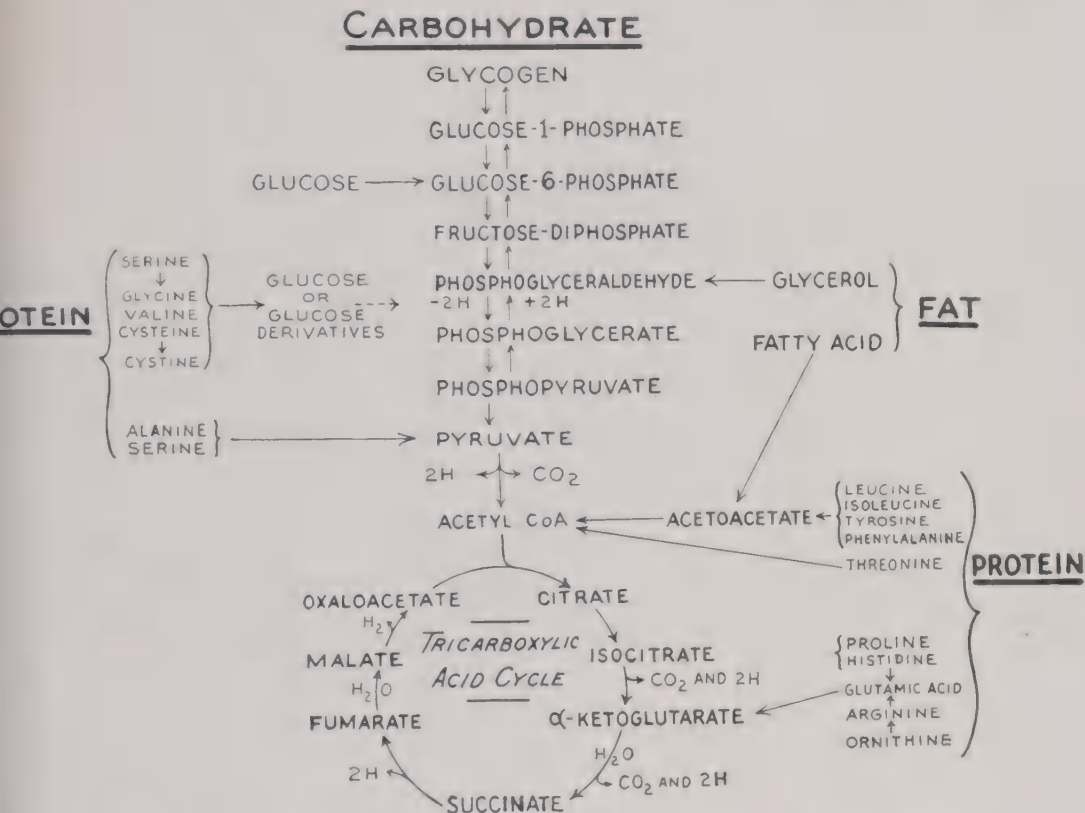


Fig. 58.—Integration of protein, carbohydrate, and fat metabolism. The scheme indicates various points at which the products of protein, carbohydrate, and fat metabolism enter into common pathways. Most of these are discussed or mentioned in the text in Chapters 15, 16, and 17.

## ESSENTIAL FATTY ACIDS

If fat is entirely excluded from the diet of rats, there develops a condition characterized chiefly by retarded growth, scaly skin, necrosis of the tail, kidney lesions with bloody urine, and early death. This was studied carefully by Burr and Burr, who found that certain unsaturated fatty acids were effective in bringing about a cure of the condition. These are linoleic, linolenic, and arachidonic. Strictly speaking, only linoleic acid is essential and cannot be synthesized by the body, and the others may replace it or spare it to some extent. Arachidonic acid is a twenty-carbon chain acid with four unsaturated linkages. Linoleic and linolenic each have 18 C's, the former having two and the latter three double bonds.

The investigation of this condition led to the discovery of the fact that in rats suffering from a lack of the essential fatty acids, the serum lipids had a low iodine number. It was soon found that human subjects with eczema likewise had serum lipids with a low content of unsaturated fatty acids (Hansen). The administration of suitable fats cleared up the skin lesions in many of these

cases (see Fig. 59). Evidently some individuals require a greater amount of these essential fatty acids in their diet than the average.

Another deficiency disease with symptoms closely resembling those described above can be produced in rats by withdrawal of pyridoxine from the diet. Apparently there is a relationship between the essential fatty acids and pyridoxine, because animals deprived of *both* the vitamin and linoleic acid can be relieved by the administration of either. The nature of this relationship is at present uncertain. (Hogan and Richardson; György; Quackenbush.)



A.

B.

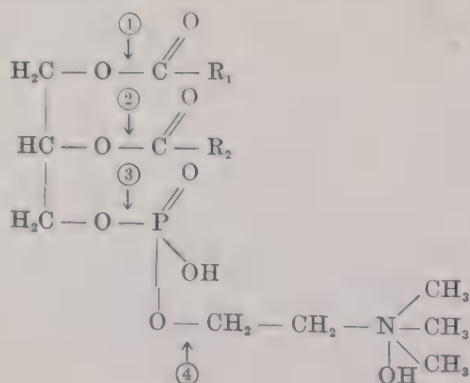
Fig. 59.—Essential fatty acid deficiency in a child 5 months of age. A, Before treatment; B, after treatment with two to three teaspoonfuls of lard daily for one month. (Courtesy Dr. Arild E. Hansen; see also Burr, G. O.: *Federation Proc.* 1: 224, 1942.)

### Rancidity of Fat

The oxidation of the volatile fatty acids during the production of rancidity results in the presence of peroxide linkages. These rancid fats then act as oxygen carriers which undoubtedly accounts for their mildly toxic effects. It has been found that they may destroy vitamin A, carotene, vitamin E, and linoleic acid. Other essential food accessories which are perhaps similarly oxidized are vitamin D, pantothenic acid, pyridoxine, and biotin.

### Metabolism of Lecithin

Lecithin can be attacked by four different enzymes in the following manner:



Lecithinase A attacks point 2, splitting off one fatty acid and lysolecithin, a hemolytic agent. This enzyme is found in animal tissues as well as in some snake venom, etc. Lecithinase B takes off two fatty acid molecules at points 1 and 2, leaving glycerocholinephosphate. It is found more generally in animal tissues. Point 3 is the spot where glycerophosphatase acts, and at point 4 choline phosphatase strikes phosphoric acid in another way. Phosphatases are also found in many tissues.

Whether or not food lecithin is digested is a question. None of these enzymes is present in any digestive fluid, except, perhaps, as a contaminant because of disintegrating leucocytes or other cells.

However, lecithins and other phospholipids get into the circulation even if they are not ordinarily digested in the gastrointestinal tract. During fat absorption the lecithin of the blood increases as the blood fat increases, and as a rule the high lecithin values persist longer. As has been seen, the phospholipids take part in fat emulsion, absorption, and fatty acid transport. Indeed the addition of lecithin increases the digestibility and absorption of fats. (Augur.) Lecithin may be synthesized by the body. Radioactive phosphorus has been converted into phosphate and fed in that form to animals. The labeled phosphorus was then found to be present in the tissue phospholipid fraction, more particularly as lecithin and cephalin, with very little as sphingomyelin. The rate of production of phospholipid, under these conditions, was found to be liver > intestine > kidney > muscle > brain. The slow production (and disappearance) in brain is interesting, in view of the high content of phospholipid in this tissue. (See page 141.) Most of the phospholipids of plasma are produced by the liver. This was shown when radioactive phosphate was injected into hepatectomized dogs. Almost no radioactive phospholipid was found in the blood plasma. Normal amounts appeared in the kidneys and small intestines, indicating that these organs can synthesize phospholipids for their own use. (Chaikoff, 1942.)

The phospholipids are very important compounds. Besides their relation to fat absorption and to the prevention of fatty livers, it should be remembered that thromboplastin, one of the important factors in blood clotting, is a phospholipid, or is associated with a phospholipid. Other metabolic functions are probable but are not yet well established.

### Metabolism of Cholesterol

Cholesterol is absorbed from the intestinal canal if there is fat absorption at the same time. Usually food containing cholesterol also contains enough fat for this purpose. Bile is necessary for cholesterol absorption just as it is for fatty acid absorption. When a neutral fat is fed to dogs or rabbits, its absorption is followed by an increase in the cholesterol esters of the blood but not in the total cholesterol (Knudson). This seems to indicate that the fatty acids are partly combined with cholesterol during absorption. In man the same rise is not seen, or it is not as marked. The cholesterol and cholesterol esters then enter the lacteals and follow the same route as the neutral fats. It is interesting to note that a highly active cholesterol esterase is present in dog serum,



but in human serum esterification goes on very slowly on incubation and is either catalyzed by an enzyme of very low activity or is nonenzymatic. (Swell and Treadwell.)

If there is an excess of cholesterol in the blood, part of it is excreted by the intestine and part by the bile. Reduction in the intestine to dihydrocholesterol and then to coprosterol by bacterial action prevents the reabsorption of this excess lipid. If a patient seems to have difficulty in handling cholesterol, a change from an animal to a vegetable diet is advisable, since plant sterols are not absorbed.

After absorption, cholesterol is converted into a variety of substances. This has been demonstrated by following the course of cholesterol- $C^{14}$  and cholesterol- $H^3$  after feeding them to rats. Ring-labeled cholesterol has been shown to be transformed into the steroid hormones 17-hydroxycorticosterone and corticosterone. (Zaffaroni.) Some radioactive  $CO_2$  is formed in a relatively short time, particularly if the terminal carbons are labeled. (Chaikoff, 1952.) However, some  $CO_2$  is also derived from other parts of the molecule. Radioactive fatty acids were recovered from adrenals, liver, carcass, and feces. Some cholesterol was found to be converted to liver phospholipid and liver glycogen, and a variable amount of activity was seen in the urine. More than half was not degraded, since it was discovered in the nonsaponifiable fraction in the feces and in the liver. In the latter case storage of cholesterol is indicated. (Kritchevsky.)

Cholesterol is essential to life but, if absent from the diet, it can be synthesized by the animal. A number of investigations have demonstrated acetate to be the carbon source for both the steroid nucleus and the octyl side chain. It is possible that acetoacetate may also be used without cleavage, and certain other compounds, including acetone, pyruvate, butyrate, hexanoate, and octanoate, may be utilized by the liver for the formation of cholesterol and also long-chain fatty acids. (Brady, 1951; Gurin.) Liver tissue from diabetic animals is also capable of converting acetone to cholesterol but not to fatty acids. From the experiments of Bloch and his colleagues it appears that both carbons of acetate are used for cholesterol synthesis. The methyl carbon is the source of carbons 17, 18, 19, 21, 22, 24, 26, and 27, and the carboxyl carbon gives rise to carbons 10, 20, 23, and 25. (Wüersch.) It is strange that, although pyruvic acid can be degraded to a 2C unit, which can be used to form fatty acids, this same unit is not available for cholesterol synthesis. (Brady and Gurin.) The esters of cholesterol with the fatty acids are normally present in the blood in a more or less definite ratio to free cholesterol. Furthermore, the fatty acids so combined are the most unsaturated of all in the blood plasma. This suggests that cholesterol acts as a special transport agent for the unsaturated acids.

Another function of cholesterol is its role as a precursor of the bile acids. This has been shown by the experiments of Bloch, Berg, and Rittenberg. In a dog, the gall bladder was anastomosed to the pelvis of one kidney in such a way that all the bile flowed into the urine. Cholesterol containing deuterium was injected intravenously. That some of this had been changed to cholic acid was

indicated by the fact that the cholic acid isolated from the "urine" contained deuterium. The organs were then analyzed, and the administered cholesterol was found in highest concentration in the lungs, next in the liver, and in smaller amounts in all other organs except the nervous system. This again emphasizes the fact that brain and nervous tissue have a slow rate of general metabolism. The cholesterol in nervous tissue, at all events, does not interchange with dietary cholesterol at appreciable rates. The cholesterol present in the "urinary" bile of this animal also contained deuterium which shows that the liver excretes some of it from the blood.

One of the types of arteriosclerosis, "hardening of the arteries," is atherosclerosis. This is a very common ailment in man, and its treatment has completely baffled clinicians. In this condition there are an abnormal deposition of cholesterol and other lipids, and a hardening, or sclerosis, due to calcification. Feeding cholesterol to rabbits produces this condition experimentally, and it is well known that a high concentration of cholesterol in the blood usually occurs in patients suffering from atherosclerosis, although many individuals with hypercholesterolemia have no evident arteriosclerosis, and not all arteriosclerotic persons have elevated blood cholesterol. Consequently there is a difference of opinion among clinicians as to whether low cholesterol diets should be advised in atherosclerosis. Unfortunately such diets are of little value unless carried out extremely rigorously. It must be remembered that the body is capable of synthesizing a considerable amount of this lipid. Keys has shown that the ordinary variations in the cholesterol content of the diet have no influence upon blood cholesterol, and moderately low cholesterol diets similarly do not reduce the blood level. Only upon diets which are almost quantitatively devoid of cholesterol do patients experience a fall in blood cholesterol, and then it is quite dramatic.

The more recent trend is to suspect that it is not the actual cholesterol content of the diet which produces atherosclerosis, perhaps not even hypercholesterolemia in itself, but rather the physical condition of the lipids in the blood. Hueper was able to produce atherosclerotic lesions by introducing large colloidal particles of foreign substances (pectin, gum arabic, etc.) into the blood stream of animals. In harmony with this is the recent work of Gofman and his group, who have studied the occurrence in the plasma of giant molecules composed of cholesterol, its esters, fatty acids, lecithin, and protein. The ultracentrifuge is used to separate blood lipoproteins into classes, depending upon the particle size and fat content. The density of these particles varies inversely with the rate of flotation ( $S_f$ ). Normal lipoproteins have a slow flotation rate,  $S_f$  10 and below. When lipid metabolism is deranged, as in atherosclerosis, they have an  $S_f$  which is higher. Figures of  $S_f$  12-100 seem to be associated with atherosclerosis or some other cardiovascular disturbance. The amount of the lipoproteins must be taken into account, as well as their  $S_f$  values. Only 10 to 15 per cent of cholesterol is contained in particles of the  $S_f$  12-100 class in human beings. These particles may often be decreased in amount by restricting dietary fat and cholesterol. Another method of shifting the lipoprotein pattern in human beings is by the parenteral administration of heparin,



or "treburon," a synthetic substance having heparin-like action. It was first observed by Hahn that after heparin administration there was an increase in the translucence of plasma, rendered hyperlipemic by high fat feeding. This clearing effect did not occur *in vitro*. If, however, such turbid plasma was treated *in vitro* with plasma from another animal which had been heparinized, the clearing phenomenon occurred. (Anderson and Fawcett.) This may indicate that a deficiency of heparin is, in part, responsible for atherosclerosis. The clearing effect is also accompanied by an increase in lipolytic activity of the plasma, which may be responsible for the phenomenon, and is followed by the rapid removal of the solubilized lipid from the blood stream. (Overbeek; Grossman.)

Another approach to this problem is indicated by the observation of Rinehart and Greenberg, who found that an arteriosclerosis develops in rhesus monkeys subjected to a prolonged pyridoxine deficiency.

### Abnormalities of Lipid Metabolism

The most common abnormality of lipid metabolism is obesity. However, as mentioned previously obesity may be and often is a normal storage of fat. Its relationship to endocrine disturbances will be considered later (Chapter 23). The formation of biliary calculi, containing varying proportions of cholesterol, has been discussed on page 241 and atherosclerosis in the section just preceding this.

There are four other pathological conditions in each of which lipids are deposited in the cells of certain tissues. Not much is known about the causes of any of them and it is quite possible that several of them may have a common etiological factor.

*Xanthoma* is a disease in which yellow nodules or flat plaques appear in the skin, especially in the eyelids. They may also be formed in tendon sheaths, bone, blood vessels, and elsewhere. They vary in size from that of a pinhead to that of a bean. It is sometimes a complication of diabetes. Many of these xanthomatous deposits have been analyzed. The results have shown that they contain a mixture of various lipids, with cholesterol frequently predominating. The blood lipid level in these cases is often elevated. Sometimes the condition occurs in the absence of diabetes, jaundice, lipoid nephrosis, or any other disease which might apparently be the cause of it. *Hand-Schüller-Christian's* disease is considered a form of xanthoma. The yellow nodules are found in the cranial and other bones. They also contain large amounts of cholesterol and cholesterol esters. This is a rather rare disease, which occurs in children. *Gaucher's disease* is a congenital condition which sometimes affects several children of the same family. It is characterized by an enlargement of the spleen and liver (splenohepatomegaly) as well as by other symptoms. Although the lipids deposited in spleen and liver are mixed, the outstanding feature of this abnormality is the presence of a large amount of kersin, a cerebroside, in them. The sugars present in those lipids peculiar to Gaucher's disease may be lactose or sucrose, or both.



s well as their monosaccharide constituents. (Parke; Woolf.) The bone and bone marrow are also involved, but no analyses of them have been made. Although ordinarily the onset is in childhood, the patient may live for many years.

Another familial and congenital disease is *Niemann-Pick's disease*. The liver and spleen are again the site of lipid deposits and are tremendously enlarged, but here the predominant constituent is a mixture of phospholipids, chiefly lecithin and sphingomyelin. It occurs in infancy and causes death within a few months.

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## Chapter 18

# MINERAL METABOLISM AND WATER BALANCE

Although the inorganic constituents of the body are only a small fraction of the total amount of the body tissue, they must not be considered unimportant. They are, in fact, becoming recognized more and more as essential cogs in the human machine. They range in amount from calcium, which makes up about 2 per cent of the average body weight, to cobalt, which is present to the extent of perhaps 0.00004 per cent, and other "trace elements" which may occur in even smaller amounts. The inorganic compounds are required for several purposes: (1) They are needed to provide a suitable medium for protoplasmic activity. The irritability of muscle and nerve cells, the permeability of cell membranes, and the normal functioning of all cells depend upon a nice balance of the diverse ions, particularly  $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{++}$ ,  $Mg^{++}$ ,  $OH^-$ ,  $HCO_3^-$ ,  $Cl^-$ ,  $HPO_4^-$ , and  $SO_4^-$ . (2) They play a primary role in osmotic phenomena. These have much to do with the flow of tissue fluids, absorption, and secretion. Many salts are also of utmost importance in acid-base equilibria. (3) Certain tissues, especially bones and teeth, have a high mineral content, which accounts for their hardness and rigidity. (4) Some mineral elements become parts of specialized physiological compounds. Hemoglobin's iron and thyroxine's iodine are examples. Other ions are essential to a number of enzyme systems. Manganese, magnesium, and potassium are examples of ions needed by enzymes in carbohydrate metabolism.

## THE MINERAL COMPOSITION OF THE BODY

Seven elements comprise from 60 to 80 per cent of all the inorganic matter in the body. They are calcium, magnesium, sodium, potassium, phosphorus, sulfur, and chlorine. They are the principal minerals in nutrition—at least from a quantitative standpoint. With an increase in age the total ash, or mineral matter, of the body increases, but a decrease in magnesium, sodium, potassium, chlorine, and sulfur occurs. This is shown in Table XXXV.

TABLE XXXV  
MINERAL COMPOSITION OF THE BODY\*

AGE	BODY WEIGHT (KG.)	TOTAL ASH (GM.)	PERCENTAGE OF TOTAL ASH							TOTAL
			Ca	Mg	Na	K	P	Cl	S	
Fetus, 6 wk.	0.88	19	28	0.9	10	7	17	8	8	79
Fetus, 7 mo.	1.16	30	23	0.8	8	7	14	10	6	69
Newborn infant	2.9	100	24	0.7	5	5	14	5	6	60
Adult	70	3000	39	0.7	2	5	22	3	4	76

\*Calculated from values given by Shohl, from Macy, I. G.: J. A. M. A. 120: 35, 1942.

Under average conditions a healthy normal man excretes about 20 or 30 m. of inorganic material daily. This consists chiefly of the chlorides, sulfates, and phosphates of sodium, potassium, calcium, magnesium, and ammonium. Normally the intake should equal the outgo, except in growth and pregnancy. In Table XXXVI is shown the amount of inorganic salts in 150 American diets. Study of these figures reveals the great variation in every element determined. Since this might be due to a divergence in the amounts of food consumed, it was also calculated to a uniform basis of 3,000 calories. Even these figures show great differences between the minimum and maximum. The question naturally arises as to whether some of these amounts are too low and others too high for optimal physiological activity, or whether only a minimum of each is required and the remainder is an unnecessary excess or a "factor of safety."

TABLE XXXVI

MINERAL ELEMENTS IN 150 AMERICAN DIETARIES\*

ELEMENTS	PER MAN PER DAY			PER 3000 CALORIES		
	MINIMUM (GM.)	MAXIMUM (GM.)	AVERAGE (GM.)	MINIMUM (GM.)	MAXIMUM (GM.)	AVERAGE (GM.)
Calcium	0.24	1.87	0.73	0.35	1.47	0.73
Magnesium	0.14	0.67	0.34	0.17	0.53	0.34
Potassium	1.43	6.54	3.39	1.63	5.27	3.40
Sodium†	0.19	4.61	1.94	0.22	4.83	1.95
Phosphorus	0.60	2.79	1.58	0.72	2.30	1.59
Chlorine†	0.88	5.83	2.83	0.83	7.26	2.88
Sulfur	0.51	2.82	1.28	0.80	2.35	1.30
Iron	0.0080	0.0307	0.0173	0.0090	0.0234	0.0174

\*From Sherman, H. C.: Chemistry of Food and Nutrition, New York, 1941, The Macmillan Co.

†Since these dietary records did not show the quantities of table salt used, the figures for sodium and chlorine cover only the amounts in the food as purchased and are very greatly below the actual intake of these elements.

It is generally felt that some excess is desirable, especially in the case of calcium, iron, and phosphorus. A diminution of the inorganic salt intake to a very low level is quite deleterious to health. Osborne and Mendel found that young rats ceased to grow on an otherwise suitable diet when the total amount of an adequate salt mixture was greatly restricted. A. H. Smith and his co-workers have extended these studies. It appears that under the conditions mentioned the bones grow somewhat, in spite of the stunting of the animal as a whole, but do not increase in weight. There is also an hypertrophy of the kidney and a polycythemia. Nevertheless, the total hemoglobin of the blood is increased because the red cells, although containing a normal percentage of hemoglobin, are smaller in size. Feeding an adequate diet with a complete salt mixture to such stunted animals changes the picture completely. They increase in size, the total number of erythrocytes diminishes, and the hemoglobin goes back to a normal value. Substitution of calcium for the complete salt mixture gives only partial return toward the normal.

Two factors which enter into inorganic salt metabolism, namely, ammonia metabolism and sulfur metabolism, have already been considered (Chapter 15). These will be taken up only incidentally in this chapter.

### Calcium and Phosphorus

Calcium is needed by all cells. It is one of the ions required for physiological balance, as mentioned previously. It is present in blood serum, about half in the ionized form, and the rest un-ionized, probably bound to protein for the most part and, to a minor degree, in a calcium-citrate complex. Normally the concentration is about 10 to 11 mg. per 100 ml. of serum.

A particular and important effect of the calcium ion is upon nervous tissue. If the ionic calcium of the blood falls, the nervous system becomes hyperirritable. This may lead to tetany. On the other hand, a high calcium content depresses nervous irritability. Hence the administration of calcium salts is indicated in the alleviation of tetany arising from low calcium. Calcium is, of course, required for bone and tooth formation. If the diet is deficient in this element either or both may suffer. This is also true if the absorption of calcium is inefficient, even in the presence of an adequate amount in the diet. Growing children, particularly, require an abundance of calcium for these as well as for all other tissues. During pregnancy and lactation there is likewise a great demand for it in the diet, to provide for the growing fetus, and for the secretion of the calcium-rich milk. The requirement for calcium ions in blood clotting need only be mentioned at this point.

The absorption of calcium is quite a variable factor. It should be remembered that calcium forms insoluble salts with a number of the anions which occur in the intestinal canal. Thus, we may find much of the calcium precipitated as the phosphate, carbonate, oxalate or sulfate, or as calcium soaps, which are also insoluble and therefore unabsorbable. This will depend on the amount of soluble calcium salts present, the negative ions, the pH, and the state of fat digestion and absorption. Calcium salts are more soluble in acid than in basic solutions. Furthermore, all food calcium does not behave in the same way. For example, the calcium of all vegetables is not uniformly absorbed, and, in some cases, the vegetables actually tend to depress the absorption of calcium from other foods. This may be due to the presence or formation of oxalates, or phosphates, or to an influence on the pH of the intestinal contents (Shields). Insoluble calcium soaps form if fatty acids are present in large amount, resulting, of course, in diminished calcium absorption. At this point it may be well to remind the student of the importance of vitamin D in aiding in the absorption of calcium and phosphorus. However, at best, normal adults absorb only small amounts of calcium, perhaps 100 mg. per day.

The excretion of calcium is partly through the kidneys but mostly through the mucosa of the small intestine. Excretion into the feces continues even when the intake is low, and accordingly a negative balance is possible. The intestinal elimination of calcium may be increased by a lack of vitamin D and diminished by a suitable amount in the diet. Since calcium levels have a profound effect upon nervous irritability, it can be appreciated that a negative calcium balance if continued long enough, would cause hyperirritability and even tetany, as well as decalcification of the skeleton. These are conditions to be guarded against in pregnancy and lactation. In these states the demand for calcium is so great that supplements of calcium and vitamin D should always be provided.



About 99 per cent of the calcium of the body is present in the bones and in the form of the complex salt previously described (Chapter 6). This is in equilibrium with the serum calcium which is kept at a fairly constant level. The bone salt and serum calcium shift back and forth as necessary to maintain this constancy. Thus the bones are continually being resorbed and rebuilt. The amount of calcium which can be absorbed from the intestine is, of course, of tremendous importance in preventing the drain on the storage of bone calcium from producing a negative balance.

Contrary to common opinion, the same reversible reaction does not occur in teeth. The adult tooth, already fully formed and calcified, is not readily subject to decalcification when the body requires calcium. This is true in pregnancy and lactation as well, and the old saying, "A tooth for every child," has no scientific justification (Schour). Disturbances of calcification are only of importance in the growing tooth. Therefore, children must have an abundance of calcium with vitamin D or sunshine to help them absorb it, for tooth as well as for skeletal development.

**The Calcium Requirement.**—The amount of calcium retained by the body depends not only upon the amount in the diet, but also upon the efficiency of absorption and upon excretion. Hence it is difficult to set an absolute standard for the calcium requirement. It is better to have an oversupply than not enough in the diet. The recommended daily allowances are: for children up to 12 years of age, 1.0 Gm.; for ages 13 to 15 years, 1.3 Gm. for girls and 1.4 Gm. for boys; for ages 16 to 20 years (or even up to 25 years), 1.0 Gm. for girls and 1.4 Gm. for boys; for adults, 0.8 Gm., but increasing to 1.5 Gm. during pregnancy and 2.0 Gm. during lactation. A quart of milk supplies about 1.2 Gm. of calcium in a readily assimilable form. Consequently a good safe rule to follow is: a pint of milk a day for every adult and a quart for every child. For other sources of calcium in foods, see page 321.

**Regulation of Blood Calcium.**—The parathyroid glands exercise a regulatory effect upon the level of calcium in the serum. Removal of these glands, experimentally, results in increased excretion of calcium in the urine and low serum calcium levels and leads to tetany and eventually death. The symptoms may be relieved by injections of calcium salt solution, but as soon as the calcium is excreted the symptoms recur. Administration of parathormone, the hormone of the gland, raises the serum calcium to a normal level and this also causes a temporary cessation of the tetany. The hormone acts by mobilizing calcium reserves from the bones. Under normal conditions the shifting of calcium from serum to bone, and vice versa, is brought about by the action of parathormone. The constancy of the serum calcium level depends chiefly on this mechanism along with the absorption of calcium from the intestine, which is influenced by vitamin D. Vitamin D is also required in calcification of bone. The action of parathormone and of vitamin D, as well as the mechanism of calcification, seems to be mediated by citrate. In 1938 Kuyper showed that the precipitate of calcium, phosphate, and citrate, which can be demonstrated in vitro, is a complex similar to that existing in bone. The

solubility of this product is increased by the presence of additional citrate and magnesium ions in the solution. Later it was found that citrate injections cause the serum calcium to become more ultrafiltrable, so that a rapid excretion of calcium takes place. The rise in serum calcium, caused by parathormone, was found to be accompanied by a rise in serum citrate, and the microscopic picture of the bone was found to be similar following both of these procedures. High doses of vitamin D also raised both serum calcium and serum citrate. These facts led Dixon and Perkins to study the enzymic mechanisms in bone. They found that the "citrogenase" system, i.e. the series of enzymes which produce citric acid in the tricarboxylic acid cycle (see page 427), is more powerful in bone than is the isocitric dehydrogenase system, which acts to decompose citric acid. Apparently, the quantity of citric acid formed has a regulatory effect. Under suitable conditions, there may be produced local increased concentrations of citric acid, which may be co-precipitated during deposition of bone salt. If the concentrations of citric acid become too high the process of calcification may be reversed and the bone salt may be solubilized. This is apparently what occurs under the influence of parathormone or vitamin D.

Whenever the serum calcium is lowered, tetany is likely to result. This occurs occasionally in the newborn infant, and sometimes in rickets and in fatty diarrhea. In the last instance, a loss of fat-soluble vitamin D accounts for a diminished calcium absorption, resulting in too low a serum calcium level for the parathyroid to compensate.

Phosphorus is ingested in many forms—phosphoproteins, nucleoproteins, phospholipids, and the various organic esters and inorganic phosphates—and it is used in the construction of the same kinds of compounds in the body, as well as in the formation of bone salt. The vital part played by phosphorus compounds in many phases of metabolism and in acid-base regulation indicates how necessary it is for this element to be supplied in sufficient amount.

Vitamin D aids in the absorption of phosphates from the gastrointestinal tract, just as it aids in calcium absorption. In the absence of vitamin D a low serum phosphorus results as a rule. In rickets there is usually a normal serum calcium with a low serum phosphorus. Other cases of rickets occur in which both calcium and phosphorus are low in the serum, or phosphorus is normal and calcium is low. The typical and usual disease, however, is characterized by low phosphorus and normal calcium. Howland and Kramer established an empirical rule for determining whether a child was rachitic or not. If the product of the serum phosphorus and serum calcium (in milligrams per 100 ml.) is below 30, rickets is present or will develop, but not if it is above 40.

The ratio of calcium to phosphorus in the food intake has an important influence on the metabolism of both elements. If either is present in inadequate amount, the other is not utilized properly, even though it be present in normal quantity. In the infant and growing child the ratio Ca:P should be somewhere between one and two, that is, the calcium intake should be equal to or up to twice as great as the phosphorus. In adult life the ratio may be below one. However, as Sherman says, "Obviously when intakes of both elements are right, the ratio



cannot be wrong." That is, from a practical nutritional standpoint the Ca:P ratio is bound to be right if the recommended quantities are present in the diet. For phosphorus this amounts to 1.0 Gm. a day for boys up to 8 years of age and for girls up to 10 years; 1.2 Gm. for boys 9 to 12 and for girls 11 to 19 years; and 1.3 Gm. for all above these ages. Again, in pregnancy and lactation a greater amount must be provided.

Phosphorus is abundant in our foods and there is little likelihood of a deficiency in this element. The same cannot be said for calcium. Most American diets are below the minimum level of calcium for safety or are dangerously close to it. Sherman states that about half of the American dietaries studied by him were below the safe level, and 16 per cent were even below the minimum requirement. If the vitamin D intake should happen to be diminished in these cases, serious consequences would result.

**Vitamin D and Parathormone.**—The relation of vitamin D and parathormone to calcium and phosphorus metabolism has been mentioned in several connections. The main action of the vitamin seems to be to increase the absorption of calcium and phosphorus from the intestinal canal. It probably also increases phosphate excretion in the urine to a small extent and in this way mobilizes calcium from bone, but this is a minor effect. (See page 619.) Irradiation of ergosterol produces a series of sterols, only one of which has antirachitic properties. This is calciferol ( $D_2$ ). The others act like parathormone. In fact, one of them, dihydrotachysterol ("A.T. 10"), has an activity so similar to the parathyroid hormone that it is quite useful in the treatment of hypoparathyroidism. It chiefly causes a phosphaturia and, to a lesser degree, an absorption of calcium from the intestinal tract; the level of calcium in the blood serum is thus raised. This compound also shares the toxic properties of parathormone. Consequently an overdose must be avoided.

**Tetany.**—A low blood calcium leads to tetany. The symptoms of this type of tetany include rapid respiration and heart rate, fibrillary muscular twitching, and tonic (sustained) or clonic (spasmodic) convulsions. Infantile tetany may be due to parathyroid deficiency but usually accompanies or follows rickets. In true rickets the calcium of the serum is not far from normal, but during healing, the return of calcium to the bones may be so rapid as to cause a fall in the serum calcium to a tetanic level. In the newborn infant, tetany occasionally occurs. This is accompanied by a low calcium and a high phosphorus. The explanation offered is that a poor renal function results in a retention of phosphorus. The high serum phosphate is thought to have a tendency to decrease the ionization of serum calcium. In osteomalacia, a bone disease, again the serum calcium is reduced, and tetany may develop. However, tetany may occur without low serum calcium. This is the case in alkalosis. Pyloric obstruction, or any other condition in which persistent vomiting occurs, results in alkalosis. The same is true of hyperventilation. When alkaline phosphates are infused into a patient, there may result both alkalosis and a diminution of serum calcium. Naturally the combined effects are quite likely to cause tetany. In these instances respiration is slowed to permit of carbonic acid accumulation to help compensate for the alkalosis resulting from a relative excess of bicarbonate.



## Magnesium

Magnesium is an essential element. Magnesium-free diets can be prepared experimentally, and animals on such diets have circulatory disturbances, increased irritability, and finally convulsions and death. Prior to the occurrence of any symptoms, the lack of magnesium prevents the synthesis of protein in animals which had been subjected to protein depletion. (Menaker and Kleiner. It is quite impossible, however, to have a magnesium deficiency on an ordinary diet. It is the essential metal in chlorophyll and, therefore, occurs in all green plants. The skeletons of some marine forms are rich in magnesium. This is undoubtedly related to the fact that sea water contains more magnesium than calcium.

It occurs in bones, muscles, and nervous tissue of man. Its distribution is very uneven, probably because it can replace calcium to some extent, and this depends largely upon the amount of calcium available. It will be remembered that magnesium can take the place of calcium in the bone salt, apatite, which continually changes in composition. Human blood serum, however, has a constant magnesium content, 1 to 3.5 mg. per 100 ml. (1.6 to 5.7 meq. per liter).

The magnesium ion is another ion which influences tissue irritability. Thus when introduced in large amounts parenterally, it is a central depressant, having anesthetic and anticonvulsant effects. These effects are completely antagonized by calcium, and this antagonism has not been explained. Curiously, however low serum concentrations of either magnesium or calcium lead to the same pharmacological effects; namely, hyperirritability and convulsions. Administration of magnesium compounds by mouth produces a laxative action (probably because of nonabsorption), and, in the case of  $\text{Mg}(\text{OH})_2$ , there is an antacid effect, as well. Magnesium ions also function in enzyme reactions, as has been seen. Hence its presence in muscle and other cells is undoubtedly of vital importance for normal metabolism.

Magnesium is excreted by way of the intestine for the most part. A fraction is eliminated by the kidneys. One of the characteristic crystal forms frequently seen in urinary sediments is the "coffin plate" crystal of  $\text{NH}_4\text{MgPO}_4$ .

## Iron

The role of iron in the body is closely associated with that of hemoglobin. Its immense importance is quite out of proportion to the total amount present in the entire body, which is the insignificant value of 3 to 5 Gm. This small amount of iron is used over and over again in the body. It is not like the vitamins or most of the other organic, or even inorganic, substances which are either inactivated or excreted in the course of their physiological functions. Very little iron is lost from the body normally and, since it is a small part of the hemoglobin molecule (about 0.3%), comparatively little is needed. Iron is also a constituent of many tissues besides blood (e.g., the myoglobin of muscle) and is essential for the composition of such catalysts as the cytochromes, peroxidase, and catalase.

Iron has been called a "one-way substance." It may be absorbed in small amounts. Any excess over and above the amount absorbed is eliminated in the feces. This cannot be considered a true excretion, but rather an oversupply, which is thus wasted. Very little is excreted in the urine—less than 1 mg. in twenty-four hours, and some is lost in the sweat. (Mitchell and Hamilton.) The amount actually secreted into the intestinal canal is negligible, and careful studies of the intake and output have never revealed any appreciable negative balances except in early infancy. Hypochromic anemias usually do not result from negative iron balances but from losses of blood, which may be very difficult to detect. Hypochromic anemias are those conditions in which there is a greater diminution in the concentration of hemoglobin than in the number of red cells, and accordingly the red cells will be paler than normally. Positive iron balances occur in growing children and in pregnant women. In both instances more iron is absorbed than is excreted, which corresponds with the need to synthesize hemoglobin for the expanding blood volume.

The absorption of iron takes place chiefly in the upper part of the small intestine. Although normally very little is absorbed, under certain conditions larger quantities may pass into the body. Following a severe hemorrhage the absorption of iron may be increased ten or twenty times, but there is usually a delay before this occurs. In hypochromic anemia iron is absorbed more than normally, and in hemochromatosis an astonishing amount may be found in the tissues. This is a disorder of iron metabolism which is characterized by large deposits, in the liver and other organs, of two pigments, "hemosiderin" and "hemofuscin," the first of which contains iron. Hemosiderin is probably derived from hemoglobin. Defective absorption of iron may result from gastrointestinal disturbances, such as achlorhydria or diarrhea, leading to anemias which readily yield to large doses of iron.

A diet low in iron is not likely to cause anemia in an adult. After hemorrhage, however, additional iron is needed to make good the loss. Consequently this must be taken into account in women during menstruation, but the amount of iron involved even here is quite small. A retention of less than 2 mg. a day is sufficient to replace the hemoglobin lost in menstruation. As stated previously, pregnancy demands additional iron for the growing fetus. When the infant is born it has a considerable store of iron for future use. This is fortunate because milk is extremely low in its content of this element. There is a supply of iron in the infant's spleen and liver, but neither is as great as was formerly believed. The amount of liver iron ranges from a negligible quantity to 60 mg. The chief location of the infant's iron is the hemoglobin of the blood. With a concentration of 22 to 23 Gm. of hemoglobin per 100 ml. this is higher than at any later period in the individual's life. During the first few weeks with a constant loss of iron, and almost no iron in the milk ingested, there is an appreciable negative balance of iron, but after the second month this balance tends to approach zero. The new iron required comes for the most part from the physiological destruction of hemoglobin (Stearns and McKinley). Premature babies or twins may be deficient in iron for obvious reasons, and anemia may result unless iron medication is given.



The recommended daily allowances of iron range from 6 mg. for children under 1 year of age to 15 mg. for youths of both sexes. The latter figure is also recommended for pregnant women. Otherwise the adult intake should be at least 12 mg. per day, despite the fact that that amount is not actually required.

The iron in foods is not all equally "available." Iron in the heme combination, it has been claimed, is not as assimilable as salts of the metal. In administering iron therapeutically, inorganic iron is probably as useful as organic, and, although ferrous iron is preferable, ferric is usually converted to ferrous and is absorbed as such. It should be emphasized, however, that only small amounts are absorbed. By giving massive doses, slightly larger quantities can be forced, but there is a regulatory mechanism which hinders unlimited absorption no matter how much is available. In hemochromatosis, the condition in which iron is retained in large amounts in the form of hemosiderin, there is also an increased amount of copper in the liver and other organs. Several investigators, notably Hahn and associates and Granick, consider this regulatory mechanism to depend upon the interesting substance "ferritin."

Ferritin is an iron-containing protein, which may contain as much as 23 per cent of iron by weight. The iron is present as micelles, or colloidal particles, composed of a ferric hydroxide-ferric phosphate complex, bound rather firmly to the protein. It can be freed of iron without denaturing the protein, and this protein, "apoferritin," is homogeneous and has a molecular weight of 460,000. Both ferritin and apoferritin can be crystallized with cadmium sulfate. Ferritin has been isolated from the bone marrow, spleen, and liver of a number of different animals and has also been found in the gastrointestinal mucosa. Experiments indicate that apoferritin may not always be present in appreciable amounts in the intestinal mucosa but is formed in response to iron feeding; that is, the feeding of iron in some way brings about the formation of the particular protein which combines with it. The apoferritin, after serving its purpose as an iron acceptor and iron donor, may then become protein reserve, to be used, probably along with other proteins, in the synthesis of new erythrocytes or for other purposes.

The iron in ferritin is in the ferric form, in contrast with that in hemoglobin, which is ferrous. As the iron of the food passes down the gastrointestinal tract it is reduced to the ferrous state, if it is not already in that state, by the gastric acidity, -SH groups, ascorbic acid, or other reducing agents in the food and secretions. This ferrous iron is absorbed into the mucosal cells of the duodenum and jejunum. The cells of the mucosa regulate iron absorption by maintaining within the cells a level of ferrous iron, governed in part by their oxidation-reduction potential. These cells possess a special mechanism for the "one-way transfer" of ferrous iron into them. Radioiron studies have shown that this mechanism, or "bloc," adjusts the uptake of iron in accordance with body needs for iron and previous iron feedings. (Hahn.) The ferrous iron is oxidized, combined with phosphate, and united with apoferritin to form ferritin. This is stored in the mucosal cell. Thus the ferrous iron of these cells is in equilibrium with the ferritin in the mucosal cells and with the plasma iron of the blood stream. The amount of ferrous iron, moving into the cell, thus



depends upon the level of ferrous iron in the cell, and indirectly upon the ferritin concentration. From the mucosa the ferrous iron passes into the blood stream, the amount being dependent upon the relative redox level of the cells, and this is related to the oxygen tension in the blood. Only ferrous iron can pass into the blood. It is then autoxidized and becomes attached to one of the globulins, called "siderophilin."



This complex is also in equilibrium with the ferrous iron and ferritin in the liver, spleen, and bone marrow. In the marrow the ferrous iron is converted to heme by combining with porphyrin, and thus the store of ferritin there has immediate use. However, the ferritin in the other tissues is, of course, convertible into the ferrous form for transport to the bone marrow for the same purpose.

If there is need for iron by the body, as, for example, following hemorrhage, the ferritin of the bone marrow, liver, and spleen will be called upon first, because these organs have the largest amounts of ferritin. Only when these major sources have been depleted and the plasma iron concentration is diminished will the mucosa be called upon for its iron. When this occurs and the "physiological saturation" of the mucosal cells with respect to ferrous ions is no longer maintained, iron can be absorbed. Thus the ferritin content of the mucosa acts as a valve, permitting the absorption of only enough iron to preserve equilibrium. This is a fortunate provision of nature, since ferric ions are rather toxic and if an excess could be absorbed rapidly it would tend to precipitate the blood proteins, the protein hormones, and enzymes.

Fig. 60 indicates these relationships in a general way. It also shows how this hypothesis accounts for the saving of iron in the catabolism of hemoglobin. When the erythrocytes have finished their life cycle, the iron is reutilized. Recent investigations, using radioactive iron, have shown that this iron from "old" erythrocytes is used in preference to storage iron. (West.) It is evident that the low absorption of iron is compensated for by the very efficient utilization of iron, as well as by the intricate mechanism of storage.

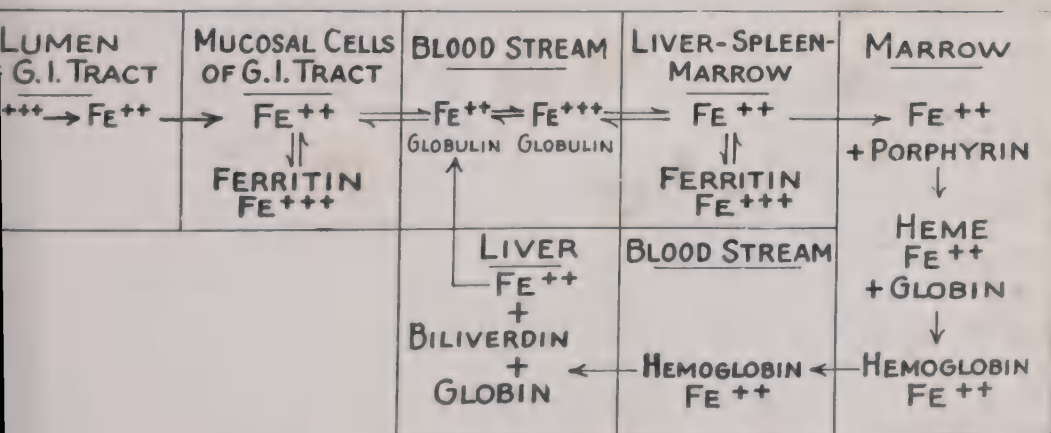


Fig. 60.—Scheme showing the role of ferritin in the absorption and storage of iron.

Ferritin has another function which seems to be quite independent of its relation to iron metabolism. Mazur and Shorr have shown that it has a vasodilator action. This is evidenced by its inhibition of the vasoconstricting effect of epinephrine. It has been identified with a hepatic vasodepressor material (VDM) which appears in the circulation in low concentration during the irreversible stage of hemorrhagic shock in animals and may prove to be the causative agent of similar hypotensive states in man. There is no ferritin in normal plasma, but in traumatic shock or liver cirrhosis traces of it appear to escape into the blood stream, causing vasodilatation and depression of the systemic blood pressure. Since ferritin may be present in tissues other than those previously mentioned, if the iron concentration of the plasma is high, it follows that injury to almost any tissue may result in its presence in the blood. Curiously ferritin has no blood pressure-lowering effect when injected into normal animals.

**Copper and Other Heavy Metals.**—For the formation of hemoglobin minute amounts of copper are believed to be needed. This is certainly the case in the regeneration of hemoglobin after dietary anemias in experimental animals and also after nutritional anemias of children. Apparently copper is used over and over again as iron is, and the loss is limited to the amount excreted in the urine and that lost in hemorrhage. The daily loss of copper in young women averages 0.04 mg., half being attributable to menstruation (Levertown and Binley). However, 2.0 to 2.5 mg. of copper per day is recommended as a daily allowance in the diet. In the male the average daily loss is probably less than 0.02 mg. Copper occurs in certain oxidases and possibly in other enzymes. It is present in many foods and the estimated daily requirement of from 2 to 2.5 mg. is usually ingested.

Copper has been found in amounts greater than normal in the brain and liver of persons dying of hepatolenticular degeneration. This is an uncommon disease of the nervous system, in which there is an associated hepatic disorder, and is generally regarded as incurable. Denny-Brown and Porter have reported high urinary copper figures in such cases. In view of this fact, they treated several cases with 2,3-dimercaptopropanol (BAL or British Anti-Lewisite) which is known to promote excretion of copper, as well as various other heavy metals and arsenic. A greatly increased excretion of copper resulted. The condition of these patients was favorably affected, sometimes to a remarkable degree.

Zinc is a constituent of carbonic anhydrase, and possibly also of uricase, and it is present in small amounts in the pancreas and human blood. (Valley) Aluminum and nickel are found in traces but, at the present time, appear to have no definite physiological function.

### Iodine

More than half of the iodine in the body is located in the thyroid gland. Thyroxine and triiodothyronine, the physiologically active substances which the thyroid manufactures, are iodine compounds, but only one-fourth or one-fifth of the total iodine in the gland is present in those forms. (See page 61) Probably most of the remainder is in the form of precursors of thyroxine. Next to the thyroid, in concentration of iodine, are the ovaries, the adrenals,

and the thymus glands. Little is known of the function of iodine except in the thyroid. The blood plasma contains from 4 to 8 gamma per 100 ml.

A lack of iodine in the food and drinking water is believed to be related to the occurrence of simple goiter. McClendon and Hathaway have shown that the drinking water in different localities in the United States varies in its iodine content from 0.01 to 73.3 parts per 1,000,000,000. Goiter occurred more frequently in persons living in those regions where the drinking water had a low iodine content. It was also shown that, in general, simple goiter is more prevalent far from the ocean, or in sections into which ocean winds cannot carry their moisture. The reason is that sea water contains iodine, and, when the sea spray is deposited on coastal regions, it enriches the soil and drinking water with this element. Vegetables grown in these regions will take up iodine from the soil, and thus the inhabitants of coastal areas will get iodine from drinking water and vegetables, as well as from sea food. Until recently goiter was common in Switzerland. Although not far from the sea, it is surrounded by high mountains which cause the ocean breezes to deposit their moisture on the far sides. As a result Swiss soil and drinking water are low in iodine. Marine and Kimball demonstrated that simple goiter could be prevented by an intake of sufficient iodine. This may be accomplished by adding inorganic iodides to the source of water supply, or more simply by insisting upon the addition of iodides to table salt. This entire matter has recently become the subject of controversy. Greenland has thrown doubt on the conception of the relationship between the incidence of goiter and the lack of iodine in drinking water and in food. This is based partly upon criticism of the analytical methods used in determining the traces of iodine and partly on other considerations. For example, the wider use of iodine in goitrous regions has not completely wiped out simple goiter in those areas, and he is of the opinion that lack of iodine is not the sole factor producing this condition. It is well known that not all goiters are due to a lack of iodine. There is for example a goiter due to infection. Exophthalmic goiter is a hyperthyroid condition which is not a result of a low iodine intake.

Iodine or iodides may be absorbed from mucous surfaces or from the skin. They are excreted chiefly in the urine, and to a minor degree in the sweat and feces. (Spector.) If given in large amounts they are also found in tears, saliva, and bile. The recommended intake is at least 0.05 mg. per day. More should be ingested during puberty, pregnancy, lactation, menopause, and when there are infections.

**Fluorine.**—Fluorine is rather widely distributed in nature and is frequently found in varying amounts in drinking water. In those localities in which the fluorine concentration is relatively high, it usually has deleterious effects upon the teeth. If it is ingested in toxic quantities during childhood while the teeth are undergoing calcification, characteristic signs appear. Instead of the normal glistening translucent appearance, the teeth acquire dull white patches, or even the entire surface may look chalky. Pitting is of common occurrence, due to the breaking off of the ends of the enamel prisms. They also may have a brown stain, a "mottling" (see Fig. 61). McCollum and others have



shown that the inclusion of fluoride in the diets of experimental animals produces fragility of the teeth and bones, and there are many other evidences that it affects calcium and phosphorus metabolism. It is an inhibitor of various enzymes, a notable example being enolase. Fluoride is sometimes added to blood which is to be analyzed for glucose, because it inhibits glycolysis.

The effect of this ion is not always unfavorable physiologically. Apparently the concentration of the fluoride present is a determining factor. This is indicated by the influence of traces of fluorides on dental caries. Armstrong and Brekhuis found that the enamel of sound teeth contained more fluorine than that of carious teeth. This is the only element which varies in this manner and it was suggested that the increased fluorine may be the effective factor in the prevention of caries. Many other observations point in the same direction. The mechanism is supposed to be that: either the fluoride actually imparts to the tooth structure caries-resistant properties, or it inhibits bacterial action on food



Fig. 61.—Mottled enamel (endemic dental fluorosis) of severe degree. Teeth calcified using water containing 14 p.p.m. of fluorine. (From Dean, H. T., McKay, F. S., and Elvov E.: Pub. Health Rep. 53: 1736, 1938.)

particles and on dental tissue. Perhaps both occur. A large scale test began in 1945 in two cities in New York. The drinking water of Newburgh has had traces of sodium fluoride (1:1,000,000) added, while Kingston, a near-by city of about the same population, is serving as a control with nothing added to its water supply. The school children in each community had their teeth examined at the beginning of the test and once a year thereafter. By 1952 the incidence of caries among the children of Newburgh had dropped 47 per cent, as compared with that among the children of Kingston. This was most pronounced in the youngest age group and was progressively less marked in the older groups. The study should give definite indications whether fluorides do or do not prevent dental caries. A positive answer may mean that fluorine is an essential element, but at present it cannot be considered as such.

**Bromine.**—Small amounts of bromine sometimes are found in table salt and also in some vegetables. Normal human serum contains about 1 mg. per

00 ml. Bromides are absorbed, distributed, and eliminated by the body in almost exactly the same manner as the chlorides. That is, bromide is absorbed from the gastrointestinal tract, passes into the various body fluids just as the chloride ion does, penetrates the red cell, but not other cell membranes, and is eliminated by the kidney. If present in sufficient amounts it tends to replace chlorine in the body, doing so in a quantitative manner. It has a sedative effect on nerve tissue, which may be a result of a decrease in the concentration of chloride, displaced by bromide in the extracellular fluid. Bromide poisoning is known as "bromism" and is fairly common, because bromides may be obtained without a physician's prescription. The advanced stages are characterized by mental and neurological disturbances.

**Manganese.**—It is now generally agreed that manganese is an essential element. It occurs rather widely in plant and animal tissues. The richest sources are liver, kidney, muscle, lettuce, spinach, and the whole grain cereals. Male rats fed on diets deficient in manganese become sterile and have testicular degeneration. The young, born of females on similar diets, do not survive long, and the mothers are unable to suckle normal young animals. These symptoms in the female may be cured or prevented by the addition of manganese to the diet. Manganese is also needed by the rat for growth (Orent and McCollum). In the chick the presence of manganese in the diet prevents the development of a condition known as "perosis"; this is an osteodystrophy. The tibial-metatarsal joint becomes enlarged, the distal end of the tibia and the proximal end of the tarsometatarsus are twisted and bent, and the gastrocnemius tendon slips from its condyles. As a result the chicks have shortened leg bones and vertebral columns. Whether deficiency in man would have results resembling those observed in the rat or in the chick cannot be said, since no case of manganese deficiency in man has been observed. Manganese is an activator of a number of different enzymes, phosphatases in particular. Other enzymes, which are more active in the presence of manganese, are phosphoglucomutase, intestinal peptidases, cholinesterase, xymase, isocitric dehydrogenase (which catalyzes the transformation of isocitric acid to oxalosuccinic acid), the carboxylases, arginase, and adenosine triphosphatase.

The exact human requirement is not known. It has been suggested that from 0.2 to 0.3 mg. per kilogram of body weight should be ingested by children daily. Probably that amount or more is regularly available. After oral or parenteral administration manganese is excreted almost entirely in the feces with extremely small quantities in the urine.

**Cobalt.**—Cobalt is an essential element for some animal species but not for others. For example, cattle and sheep in certain regions develop a peculiar disease characterized by emaciation and anemia. This has been traced to a deficiency of cobalt, and the administration of cobalt is effective in the treatment of the condition. Horses grazing on the same lands remain healthy. A slight excess of cobalt in either metallic or ionic form produces polycythemia (excessive formation of red cells) in rats and in a number of other species. Rats on a copper-deficient diet fail to develop this cobalt polycythemia, and in a number of other

respects copper seems to be related to cobalt in the animal body. It has also been claimed that nickel and cobalt are required for the normal functioning of the pancreas.

Since cobalt is a constituent of the vitamin B<sub>12</sub> molecule, it is evidently necessary for hemoglobin formation and must be regarded as essential. Human foods containing over 0.2 part per million include buckwheat, figs, cabbage, lettuce, spinach, beet greens, and water cress, and there are smaller quantities in other vegetable and animal products. It is also a contaminant of many medicinal preparations of iron.

**Other Trace Elements.**—Among the other elements which have been found in traces in animal tissues is selenium, which is present in the soil and plants of certain localities. Animals feeding in these regions may acquire the “alkali disease.” This element does not appear to have any beneficial effects for animals or man. Lead is found in some foods and especially in drinking water. It is stored in the bones and, to a less extent, in the liver. In large amounts it is toxic. Its deposition in bones may be explained by postulating that lead, phosphorus, and vitamin D form a system of lead deposition analogous to the deposition of calcium in bones. (Sobel.) Tin also occurs in the body; the largest quantities are found in the tongue and skin. In the concentrations usually occurring in foods, as a result of their having been preserved in tin containers, this metal has no deleterious effects. Silicon, as silicates, enters the body chiefly in vegetable foods. Soluble silicates are easily absorbed. Human blood serum ordinarily carries about 1 mg. per 100 ml. After ingesting silicates this level does not rise because the excess is rapidly excreted by the kidneys. Varying quantities are found in the different organs and tissues. The lungs are highest in silicon because of the inhalation of insoluble particles which lodge there. In industries in which silica dust is produced in large amounts, e.g., stonecutting, the workmen inhaling this dust develop “silicosis.” In this condition the lung tissue is replaced by nodular connective tissue overgrowths. Naturally the silicon content of such lung tissue is comparatively high. Similar pathological states result from breathing dusts of other types—coal, steel, etc. Other elements present in traces in foods and in body tissues are boron, molybdenum, arsenic, and titanium.

**Summary of Trace Elements.**—Six elements which are found in very small amounts in food are essential for mammalian life and health. They are iron, iodine, copper, manganese, zinc, and cobalt. Only iron and iodine are likely to be deficient in the diet.

### Sodium, Potassium, and Chlorine

Sodium chloride is added to food in cooking and at the table in an amount greater than is usually present in the uncooked food. It is the only salt which is added to the diet and is needed for both its positive and negative ions. This requirement is shared with the herbivora. They do not obtain sufficient sodium from plants, which are rich in potassium; consequently they seek out deposits of sodium chloride, the so-called “salt licks.”



In the body, sodium ions predominate in the plasma and other body fluids, while potassium occurs to a greater extent within the cells, both of the blood and tissues in general. A sudden increase of potassium salts in the diet experimentally causes an immediate increase in sodium and chlorine elimination in the urine for twenty-four hours. Since many foods are higher in potassium than sodium, this might easily occur and result in a subnormal sodium content of the body were it not for the addition of NaCl to our food. However, if the high potassium intake is continued for several days, the NaCl output is diminished even to a point below the amount ingested (Miller).

Sodium chloride and other salts aid in keeping the serum globulin in solution, and they function in the various other ways mentioned earlier. For example, an excised heart will continue to beat for hours if, under suitable conditions, it is perfused with an oxygenated solution of salts. The optimum concentrations of these salts vary with the type of animals, but in all cases sodium, potassium, and calcium must be present. Calcium and potassium seem to be antagonistic to each other in such a nutrient solution. The required osmotic pressure is produced chiefly by the predominance of NaCl, but the sodium ion itself is essential and, of course, the pH must be suitable. An example of such a solution is Locke's, which contains 0.92 per cent NaCl, 0.042 per cent KCl, 0.018 per cent  $\text{NaHCO}_3$ , and 0.1 per cent glucose.

More sodium than potassium is needed by the body. The usual daily intake of sodium chloride is about 10 or 15 Gm., or 170-256 meq. This is far greater than is required, but this amount is used chiefly because of its flavor. About 98 per cent is eliminated by way of the urine and 2 per cent in the feces. The usual amount of potassium in the diet, on the other hand, is only 2 to 4 Gm., or 50 to 100 meq. per day. Table LIII (Appendix) shows the sodium and potassium content of many foods.

Although loss of fluid and loss of salt generally accompany each other, a deficit of NaCl alone may be encountered. The symptoms are weakness, fatigue, lack of appetite, nausea, and a diminution of mental acuity. Impairment of renal function with delayed diuresis follow. (McCance.) A thirst develops which cannot be allayed by drinking. Salt, however, does alleviate it.

Chlorine is an essential anion. It is closely connected with sodium in foods, body tissues and fluids, and excretions. It has been seen how it is needed in the "chloride shift" and the formation of gastric HCl. Chloride is excreted, mostly as NaCl, and chiefly by way of the kidney. About 1 per cent is eliminated in the feces, and perhaps 4 or 5 per cent in sweat.

Ordinary diets contain sufficient sodium, potassium, and chlorine, but when there is excessive excretion of any of them, more must be provided. Adrenal insufficiency and acidosis are examples; diarrhea and excessive perspiration are others. Men working in industries in which they encounter intense heat and perspire freely must have salt supplied with their drinking water to make up for this loss of electrolyte.

Although some sodium is found within the cells—particularly those of cartilage and muscle—potassium occurs there in much higher concentration. No other cation can entirely replace potassium for the performance of a great num-

ber of cellular functions. It must, therefore, be said to be an indispensable element. Potassium can move in and out of most cells more easily than sodium according to the demands of shifting membrane equilibria. Probably changes in acid-base balance influence these shifts considerably. Under normal conditions the respective concentrations of  $\text{Na}^+$  and  $\text{K}^+$  are held within a fairly narrow range although, as shown by tracer studies with radioactive isotopes, these ions move freely across cell membranes.

In the building of cells the potassium ions are taken up; this appears to be essential for growth. In infancy and childhood and during pregnancy and lactation there is a comparatively high potassium retention. During muscular contraction there is a loss of potassium from the muscle cells to the extracellular fluid. (Fenn.) Subsequently this lost fraction returns to the muscle tissue. The significance of this movement of potassium during muscle contraction is unknown, but it seems to be connected with the contractile process rather than with the neuromuscular transmission of the stimulus. In the steady state it is probable that the loss due to contraction is just equal to the gain due to recovery. Undoubtedly this is the condition in cardiac contraction, for potassium ions are essential for heart rhythm.

Potassium also is necessary for nervous activity and the same type of movement of the ion occurs here. Nerve fibers are exceptionally rich in this element. When the nerve is stimulated, potassium diffuses into the surrounding fluid very rapidly, and during rest it diffuses back. This diffusion seems to be associated with a change in potential which occurs during the conduction of the nerve impulse, but its exact physiological role is not known.

It is now known that potassium is related to carbohydrate metabolism. The potassium level of the plasma rises and falls with the lactic acid level and with the concentration of blood sugar. It falls after insulin administration and rises after giving epinephrine. Glycogen formation from either glucose or pyruvate requires potassium ions. The exact manner by which these ions influence glycogenesis in liver has not been identified, but it is felt that the maintenance of a normal intracellular ionic environment is essential. Other ions probably needed are magnesium, calcium, bicarbonate, and chloride (Fenn; Hastings.) Since glycogen deposition in the liver is accompanied by the deposition of potassium, the administration of insulin may, under certain conditions, tend to shift potassium from the extracellular fluid into the cells. In diabetic acidosis, apparently the failure to metabolize glucose properly is associated with loss of potassium from the cells. There follows an increased excretion of potassium in the urine if the kidneys are functioning efficiently. Often there is vomiting, with further loss of potassium. However, the plasma level of potassium is usually not below normal, because the urinary excretion cannot keep pace with the influx of potassium from the cells. When insulin is administered, the extracellular fluid potassium will be shifted into the cells, and a hypopotassemia will occur. This may lead to several alarming symptoms, including paralysis of the respiratory muscles. (Holler; Sprague and Power.) Hence, under such circumstances the replacement infusion fluid should contain potassium. (See Table XXXVII.)



In certain types of hypertension rigid restriction of sodium in the diet has been found by some investigators to have a beneficial effect. (See page 335.) This is a very controversial subject, but in this connection the findings of Sapirostein and Greene are interesting. In hypertensive rats the sodium content of the entire body was elevated, while potassium remained unchanged. Their data indicated a penetration of the intracellular compartment by sodium. If this occurs, it must be because sodium displaces some other intracellular cation or is in an osmotically inactive state. Other interrelationships of sodium, potassium, and chlorine will be taken up later in this chapter in connection with water balance.

## WATER BALANCE

The study of water regulation in the body has made great strides in recent years. As might be expected it is clearly bound up with sodium and potassium distribution, although other factors also are concerned. Among these are acid-base equilibrium; the intermediary metabolism of the proteins, carbohydrates, and fats; some of the hormones; and certain physical factors.

### Pathways of Salts and Water

The necessity for the various salts has been discussed in the first part of this chapter. They must, of course, be in solution in order to be absorbed. The water is derived from water and other beverages drunk and from the water content of solid foods. Most of the absorption is through the mucosa of the upper intestine. Besides the water actually present in food and drink, a small amount is produced in metabolism by the oxidation of the hydrogen of the metabolites. The amount of this will vary but is generally thought to be from 300 to 350 Gm. According to Magnus-Levy, 100 Gm. of fat yields 107 Gm. of water; 100 Gm. of starch, 55 Gm. of water; and 100 Gm. of protein, 41 Gm. of water. The water absorbed goes first into the interstitial fluid; that is, the lymph and tissue juices. From here it passes into cells or blood plasma and wanders back and forth, depending upon conditions. Eventually it is excreted by four channels—the skin, the lungs, the kidneys, and the intestines. Salts accompany the water into the sweat, urine, and intestinal secretions.

A typical balance for an average-sized man might be as follows:

WATER INTAKE	GM.	WATER OUTPUT	GM.
Drinking water	400	Skin	500
Water in other beverages	580	Expired air	350
Preformed water in solid foods	720	Urine	1,100
Metabolic water	320	Feces	150
	<hr/> 2,020		<hr/> 2,100
			Balance = -80 Gm.

### General Distribution of Body Fluids

The total amount of fluid in the body is about 70 per cent of the body weight. About 5 per cent of the body weight is blood plasma, roughly 3.5 liters in a person weighing 70 kilograms. (It will be remembered that the blood makes



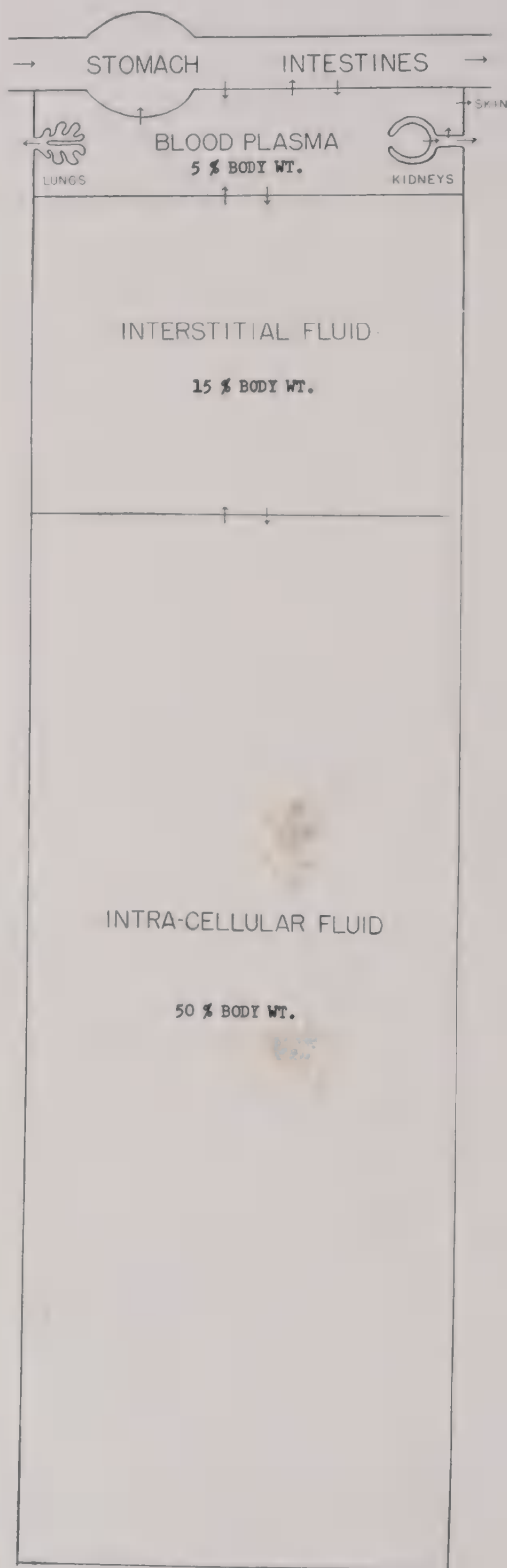


Fig. 62.—The distribution of body fluids. (Modified from Gamble, J. L.: Chemical Anatomy, Physiology and Pathology of Extracellular Fluid, ed. 5, Cambridge, Mass., 1950. Harvard University Press.)

p about one-twelfth of the body weight, or  $8\frac{1}{3}$  per cent, and about 60 per cent of this is plasma; i.e., 5 per cent of the body weight.) The lymph and other extracellular fluids, or "tissue juices," comprise the interstitial fluid and total about 15 per cent of the body weight, or 10.5 liters. The intracellular fluid is estimated at 50 per cent; in this case, about 35 liters (Fig. 62). The figure for intracellular fluid is an estimate based on determinations on animals. The other values have been obtained by experimental methods. The estimation of total body water presents an interesting problem. Most of the procedures have aimed at injecting intravenously a substance which would distribute itself uniformly in the total body water and then determining its concentration. It must be a harmless substance which would not be destroyed quickly. Most of the substances tested have been unsuitable because of their uneven distribution among the different tissues. Recently, however, "heavy" water, deuterium oxide (Moore) and "tritiated" water (Pace) have been successfully employed. The latter contains a small fraction of tritium, the radioactive isotope of hydrogen of mass 3. These "heavy waters" are ideal for this purpose because, although they differ enough from ordinary water to permit their determination when mixed with it, they are handled by the body exactly in the same way as ordinary water. A few milliliters of tritiated water is injected after determining its radioactivity. At different intervals samples of blood are removed and the radioactivity of the plasma is measured. By taking the average after one, two, and three hours, the figure of 64.7 per cent of body weight was obtained for the total body water in one human subject, as compared with 65.2 per cent obtained by calculation by other methods. The error is about 10 per cent.

It is the interstitial fluid which is the "middleman" of the body fluids. This is the medium through which nutrient materials pass from the blood to the cells and sometimes in the reverse direction. Through it also waste products travel from the cells to the blood. Its hydrogen ion concentration and osmotic pressure must be in equilibrium with both the plasma and intracellular fluids. It shrinks or expands in volume easily as the various physiological functions add to or subtract from the body water. In rapidly occurring pathological disturbances of fluid balance, the total interstitial fluid may fluctuate tremendously, and thus it protects both the blood volume and the cellular fluid from sudden change. In this manner the interstitial fluid is instrumental in preserving a normal constant equilibrium, a "homeostasis." When extreme fluid loss occurs, plasma fluid is the second to be depleted. The intracellular fluid is the most vital and is preserved to the end. However, when the fluid loss is gradual, as in water deprivation, all three compartments suffer equally.

### **Electrolyte Content of Body Fluids**

The concentrations of electrolytes in the three "compartments" of fluid, as they are called, are maintained within narrow ranges during health. The cells are the last to suffer any changes in electrolyte concentration, as they are in water; the interstitial fluid and blood plasma bear the brunt of any fluctuations.

## ACID-BASE COMPOSITION OF BLOOD PLASMA

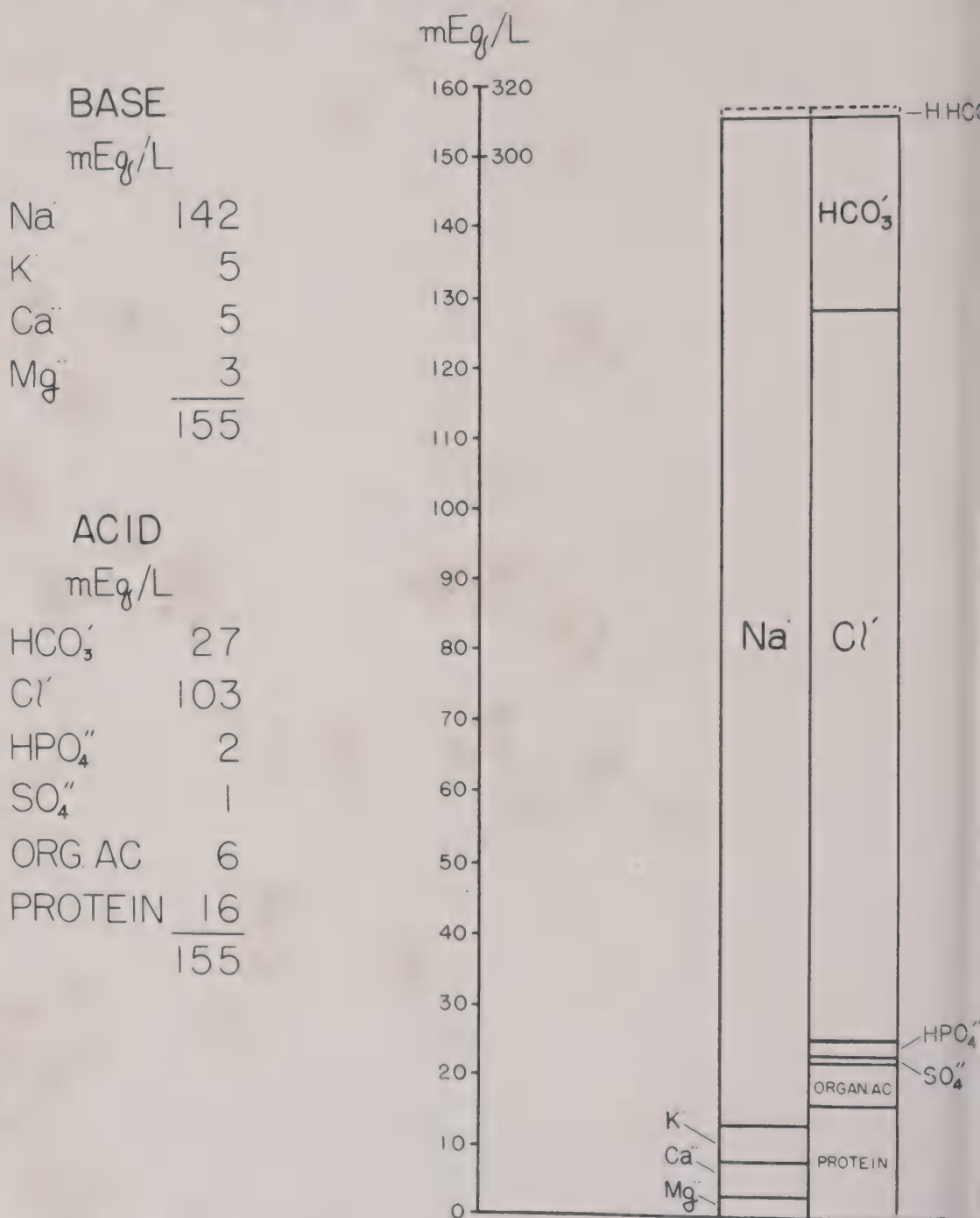


Fig. 63.—Acid-base composition of blood plasma. (From Gamble, J. L.: Chemical Anatomy, Physiology and Pathology of Extracellular Fluid, ed. 5, Cambridge, Mass., 1950. Harvard University Press.)



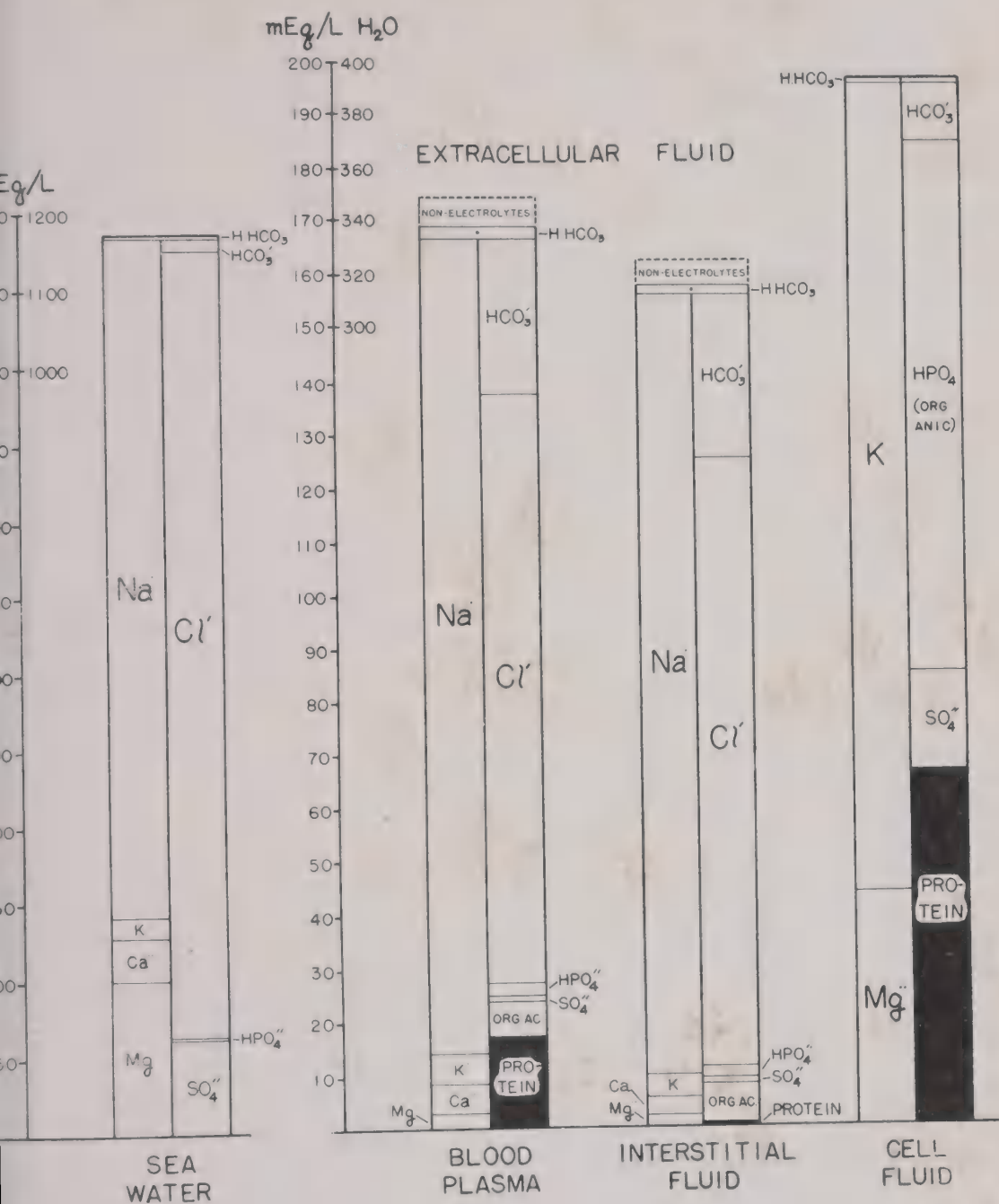


FIG. 64.—The chemical composition of extracellular fluids and of sea water and cell fluid. Note that the values are given as milliequivalents per liter of water contained in the fluid instead of per liter of plasma, as in Fig. 63. It will be seen that the patterns of blood plasma and interstitial fluid are almost identical; the greatest single item of difference is in the amounts of protein. This makes necessary adjustment of the concentrations of the diffusible ions which will preserve the total cation-anion equivalence (Donnan equilibrium). The non-electrolyte concentration (glucose, urea, etc.) is seen to be very small in comparison with that of the electrolytes although the total quantity carried to the tissue cells and into the urine over a unit of time is several times larger. The history of extracellular fluid is clearly indicated by the resemblance of its chemical pattern to that of sea water, which is roughly three times more concentrated than plasma. Note the predominance of potassium and the high protein content of cell fluid. (From Gamble, J. L.: *Chemical Anatomy, Physiology and Pathology of Extracellular Fluid*, ed. 5, Cambridge, Mass., 1950, Harvard University Press.)

The electrolyte composition of the blood plasma is shown in Fig. 63.

The values are expressed in milliequivalents per liter. A milliequivalent weight is one thousandth of an equivalent weight. Milliequivalents per liter may be calculated from the number of milligrams per liter by the following formula:

$$\text{Milliequivalents per liter} = \frac{\text{Milligrams per liter} \times \text{Valence}}{\text{Atomic weight}}$$

Thus a milliequivalent of any one element or ion is equivalent to a milliequivalent of any other. The fact that there are 5 meq. of potassium per liter and 5 of calcium in plasma conveys the idea instantly that these two are of the same relative value as bases, whereas the figures 200 mg. K per liter and 100 mg. Ca per liter would not.

Venous plasma has approximately the same electrolyte composition as arterial, except for the bicarbonate which is higher. Interstitial fluid probably has very nearly the same inorganic composition as plasma; however, because of a lower concentration of protein than in plasma, the distribution of the diffusible ions will be somewhat different as a result of a Donnan equilibrium effect. Partly for the same reason the distribution of the diffusible ions in the cells is also different, since the nondiffusible protein in the cells is much higher than in the interstitial fluid (see Fig. 64). It will be noted that sodium and chloride are the predominant ions in the extracellular fluids, but potassium and phosphate are the major intracellular ions. Protein contributes its important "colloidal osmotic pressure." (See page 169.) Bicarbonate, which occurs in all three compartments, fluctuates considerably as it is formed and excreted constantly, and both bicarbonate and phosphate contribute to the regulation of acid-base balance.

### Intake of Water

The total amount of water absorbed by the body depends upon a number of factors. In health an important influence is the external temperature, because water is concerned in the regulation of body temperature. Ordinarily a normal person satisfies his requirements by the ingestion of food and drink in moderate amounts and does not experience the sensation of thirst. Thirst appears in health when there is an inadequate amount of water in the body. While the exact cause of thirst is not known, it appears to depend on decreased water content and possibly increased osmotic pressure of the cells. Usually it is alleviated by drinking water. However, thirst associated with dehydration probably is a sign of a lack of salt as well as water. Men cannot discriminate between salt hunger and water hunger as animals can. It is therefore important under some conditions to provide salt with the drinking water to replenish the electrolytes of extracellular fluid.

### Output of Water and Salts

The loss of water and salts by way of the skin, lungs, and intestinal canal is governed by physiological needs. The excretion through the skin and lungs is chiefly a matter of heat regulation and bears little relation to the intake of fluid. The water secreted by the intestine is the solvent for excretory products

and is needed to insure suitable consistency to the feces. Renal secretion normally is highly flexible. If a large amount of water has been ingested or produced, the kidney excretes the excess. If the water intake is low, this organ can and does produce a concentrated urine so that little water is lost from the body. Similarly the kidney can conserve or eliminate salt, depending upon dietary intake.

The inspired air at ordinary temperatures contains very small amounts of water. Expired air, on the contrary, is almost saturated. The familiar condensation of moisture after breathing on a cold glass object is evidence of that. Consequently any increase in pulmonary ventilation will increase the water loss by this pathway. The evaporation of water from the lungs is one of the body's methods of losing excess heat.

**Sweat.**—Sweating is an important means of getting rid of body heat, since heat is used in evaporation. At moderate temperatures this evaporation keeps pace with secretion, and no actual drops of sweat form. This is called "insensible" perspiration. With higher temperatures the sweat glands become more active and secrete more freely. Evaporation is faster, unless the humidity of the air is high. Sweating is accelerated also, for the purpose of dissipating heat, when there is considerable muscular activity. Therefore the amount of perspiration normally secreted will depend upon the temperature and relative humidity of the atmosphere and upon the muscular activity of the individual. The insensible perspiration range is from 300 to 600 ml. per day; sensible perspiration may be any additional quantity.

It is of course difficult to obtain sweat for analysis, but by the employment of microchemical and microbiological methods our knowledge of its composition is becoming more complete. It has a specific gravity of about 1.002 to 1.003, with a pH reputed to be anywhere from 5.2 to 7.3. Urea is present in concentration four or five times that of blood. Glucose is present in minute amounts, much less than in blood. Ten free amino acids have been found by microbiological methods. Most of them are in about the same concentration in blood, but four are much higher in sweat—arginine and histidine are about 10 times higher in sweat. (Hier.) Sweat is said to have about one-fifth as high a concentration of sodium chloride as blood plasma. It is therefore evident that many of the constituents of sweat are not merely a result of filtration from the blood plasma. Usually less than 0.1 Gm. of nitrogen is secreted in the perspiration each day, but if sweating is profuse, as much as 0.2 Gm. may be eliminated in a single hour. Besides NaCl, there occur some potassium salts and appreciable amounts of calcium, magnesium, phosphorus, and iron, and traces of copper and manganese. (Mitchell and Hamilton.)

**Gastrointestinal Secretion of Water.**—The water which leaves the body by way of the intestinal canal is small in amount under ordinary circumstances because the water of the digestive fluids is largely reabsorbed along with the water of the food and drink. Some materials are actively secreted and must be held in solution, and the feces must not be permitted to become too hard and dry. When diarrhea or vomiting occurs, large amounts of water and electrolytes may be lost, especially Na, K, H, Cl, and  $\text{HCO}_3$  ions. The



gastrointestinal secretions contain potassium in concentrations higher than those of extracellular fluid, although lower than those present within the cells. Consequently the loss of potassium by this route is of great importance, since it may lead to grave potassium deficits.

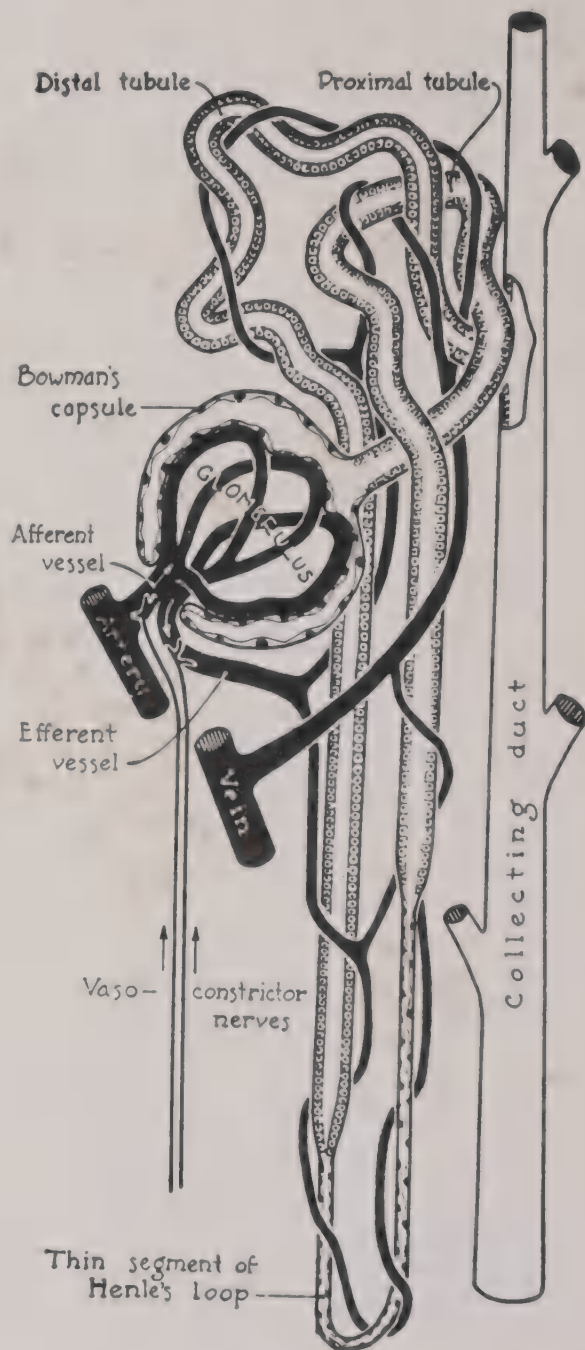


Fig. 65.—Diagram of a single nephron. (From Amberson, W. R., and Smith, D. C.: *Outline of Physiology*, Baltimore, 1939, Williams & Wilkins Co.)

**Secretion of Urine.**—The water-excreting function of the kidneys is by no means their only function. They excrete waste products, aid in acid-base regulation both by excreting acids or bases and by producing ammonia, and, in

addition, probably produce physiologically active substances. At the moment we are concerned with their activities in excreting water and salts.

The functional unit of the kidney is the "nephron," which consists of a glomerulus, a convoluted and collecting tubule, and blood vessels (Fig. 65). The afferent arteriole goes to the glomerulus, carrying blood to the tuft of capillaries which supply it, then continues on as an efferent arteriole. It now breaks up into capillaries again which are the sole blood supply of the tubules. The afferent arteriole enters the glomerulus, and the glomerular filtrate is formed. This is believed to be a fluid, very low in protein, formed by the process of ultrafiltration. The concentration of solutes in the glomerular filtrate is similar to the arterial plasma except for the protein. As a result of this filtration the blood is more concentrated as it leaves the glomerulus in the efferent arteriole. Its protein content is consequently increased and therefore its osmotic pressure is greater. It is this blood which surrounds the tubules as the glomerular filtrate flows through them. Most of the filtrate is now reabsorbed through the tubule cells. About 90 per cent of the water is reabsorbed as a result of difference in pressure. Glucose is reabsorbed after enzymic phosphorylation in the tubular epithelial cells. Sodium, potassium, amino acids, and other substances are also reabsorbed, perhaps by enzyme mechanisms. Carbonic anhydrase controls the carbon dioxide transfer. Urea, creatinine, uric acid, and certain other compounds are not absorbed proportionately to the fluid. At the same time certain solutes are added to the fluid by an excretory function of the tubules. Among these are hippuric acid, other derivatives of benzoic acid, and still other organic waste products. Of the 10 per cent of water remaining, much is reabsorbed as a result of the action of the antidiuretic hormone elaborated by the posterior lobe of the pituitary gland, except for 1 or 2 per cent. This small fraction, this 1 or 2 per cent of the glomerular filtrate, flows into the urinary bladder as urine. Since an adult excretes from 1,000 to 1,800 ml. in twenty-four hours, it is evident that the total glomerular filtrate must be in the neighborhood of from 50,000 to 180,000 ml. The end result, urine, is generally a fluid having an osmotic pressure greater than that of the body fluids. In this way the kidney conserves water. However, the kidney may also produce a more dilute urine. The tubular cells may actively reabsorb most of the solutes of the glomerular filtrate. This occurs, for example, after the ingestion of large quantities of water and leads to "water diuresis" with the formation of an extremely dilute urine.

The plasma electrolytes pass through the glomerulus, but some potassium leaves the blood by way of the tubules, perhaps by active secretion. (Berliner and Kennedy; Mudge.) Sodium and chloride ions predominate in the glomerular filtrate just as they do in the plasma. As this flows through the tubules they are reabsorbed into the blood along with the water. The electrolytes, like the water, are not absorbed completely. Part of the absorption of NaCl is brought about through the influence of a hormone of the adrenal cortex, desoxycorticosterone. This is the more particular site of the action of this hormone.

Diuresis, the increased secretion of urine, is brought about chiefly by a failure of the tubules to reabsorb their usual quota of the glomerular filtrate.

Sodium salts and urea exert diuretic effects. After they filter through the glomerulus, they increase osmotic pressure to such an extent that less filtrate is reabsorbed by the tubules. There are some diuretics such as organic mercurial compounds which may exert their action on the enzymes in the tubular cells, preventing the absorption of sodium salts and hence of water.

**Dehydration.**—Dehydration may result from an inadequate intake or an excessive loss of water, or both, and is of two types: (1) a dehydration due to deprivation of water alone and (2) that resulting from a pathological loss of water and electrolytes (Fig. 66). The output of water may be due to diuresis, or to a loss of water from the gastrointestinal tract as a result of diarrhea, or, more frequently, persistent vomiting. There are all degrees of dehydration, from a mild state to an exceedingly severe one which may establish itself more rapidly than one would believe possible. At first the interstitial fluid suffers a shrinkage and not much harm is done, and if water is taken by mouth, there is a restoration of normal conditions. Losses of the second type require calculated replacement of electrolytes and water.

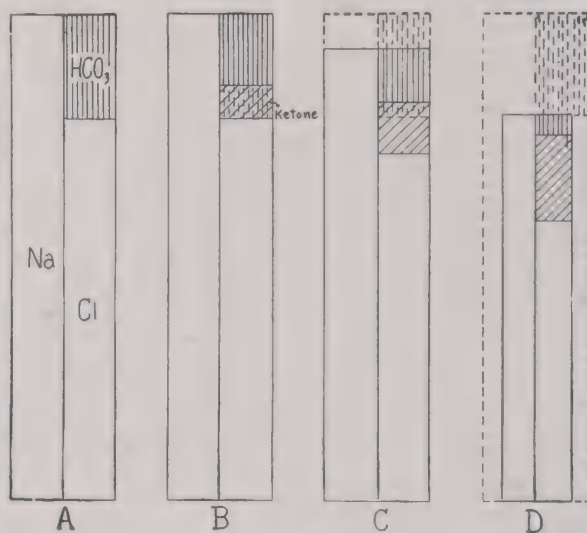


Fig. 66.—A schematic representation of the effect of diabetic acidosis on the volume and composition of extracellular fluid. The vertical dimension represents osmolar concentration and the horizontal, volume of fluid. For purposes of simplification, only Na, Cl,  $\text{HCO}_3$  are depicted. The broken lines in each case indicate the pattern of normal interstitial fluid for comparison.

The beta-hydroxybutyric and diacetic acids in excess displace the bicarbonate as shown in B. This results in the excretion of some sodium as salts of these acids and the elimination of  $\text{CO}_2$  by the lungs. For some unknown reason NaCl is also excreted in large amounts. These salts take water along with them and dehydration is accelerated. The chloride continues to be excreted even after its concentration in the blood serum is greatly diminished, C. The patient experiences extreme thirst but is unable to retain water by mouth because of nausea and vomiting. In fact, these add to the salt depletion and dehydration. In the attempt to excrete  $\text{CO}_2$  through the lungs, overventilation occurs which takes away more water. The final stage is suggested by D. Here the dehydration is caused in part by the high glucose in the blood, which is diuretic. The diacetic acid and beta-hydroxybutyric acid are buffered by blood bicarbonate, thus lowering the bicarbonate. They also must be excreted. Despite an increased formation of ammonia by the kidney to help neutralize these acids so that they can be excreted, they take away some sodium into the urine. Consequently there is a loss of fixed base from the blood along with a lowered bicarbonate. (From Peters, J. P., in Duncan, G. G.: *Diseases of Metabolism*, Philadelphia, 1942, W. B. Saunders Co.)

## PATHOLOGICAL DEHYDRATION AND RELATED CONDITIONS

At this point it may be well to repeat the statement that normally the total osmotic pressures of the plasma, of the interstitial fluids, and of the intra-



cellular fluid are all the same. This does not hold for secretions, such as sweat and saliva, which are secreted onto relatively impermeable stratified epithelium, but it does hold for all truly internal fluids. The osmotic pressure is due to non-electrolytes, such as glucose and urea in some measure, and to proteins in a very minute degree, but most of the osmotic pressure is attributable to the inorganic ions. Consequently, gains or losses of electrolytes, especially  $\text{Na}^+$  or  $\text{K}^+$ , or changes in their concentrations, are usually followed by shifts of fluid which restore osmotic equilibrium.

The volume of blood in an adult's body is roughly 5 liters, of which about 1.5 liters are plasma. It is, of course, from this blood plasma that all secretions, as well as the interstitial fluid, are derived. As an example of the effect of loss of secreted fluid upon water and salt balance, McCance gives the following illustration. Assume that 500 ml. of mixed jejunal and ileal fluids have been secreted and lost from the body. A mixture of equal parts of these two secretions resemble blood plasma in composition except that they contain less protein. Consequently, this is like removing 500 ml. of protein-free plasma. The results to be expected are

- (a) A reduction in plasma volume from 2500 to 2000 ml.
- (b) A reduction in total blood volume of 500 ml., i.e., from 5000 to 4500 ml.
- (c) A rise in red blood cell count because of blood concentration.
- (d) An increase in the concentration of plasma proteins by 20 per cent, with a rise in colloidal osmotic pressure.
- (e) No change in the *concentration* of the plasma electrolytes and hence little change in the total osmotic pressure.

This would result in no change in the size of the body cells because of the constancy of osmotic pressure. However, additional losses of other body fluids would have other effects. Such losses are caused by longer periods of dehydration, pyloric stenosis, intestinal obstruction, sweating, trauma, and severe burns.

If dehydration and loss of extracellular electrolytes is continued, the volume of the blood plasma decreases, and it is found to have become concentrated. Serum proteins increase in concentration. Blood urea rises, and a negative balance of nitrogen and potassium occurs, which indicates that a generalized tissue disintegration has set in. Since the cells contain potassium, this element thus gets into the interstitial fluid and thence into the plasma and is excreted in the urine. However, prolonged dehydration from any cause has been shown to result in a greater loss of intracellular potassium than can be accounted for by protein catabolism. (Elkinton and Winkler.) A continued dehydration with concentration of blood and loss of base eventually leads to death.

Pyloric stenosis or obstruction results in excessive loss of fluid by vomiting. The fluid lost is gastric secretion which is a varying mixture of  $\text{NaCl}$ ,  $\text{KCl}$ , and  $\text{HCl}$ . Therefore, there will be a drop in the  $\text{Cl}^-$  of the plasma. There is a compensatory rise in  $\text{HCO}_3^-$  (derived from  $\text{CO}_2$ ) to preserve electrical neutrality. Plasma potassium may also be reduced if this ion is not included in the replacement solution.

Darrow has shown that in diarrhea in infants there is a decrease in extracellular water due to a loss of sodium, chloride, and bicarbonate in the watery stools. This is derived from the alkaline intestinal secretions, particularly pancreatic juice and bile. (See Fig. 67.) As sodium leaves the plasma and interstitial fluid, potassium salts move out of the cells. As a result, intracellular potassium is lost in tremendous quantities. For this reason potassium salts are added to therapeutic solutions containing sodium salts. (See Table XXXVII.) Since heart block is produced when potassium rises to a certain level in the plasma, care must be exercised in the intravenous administration of such fluids; hence oral administration is recommended. (Darrow.)

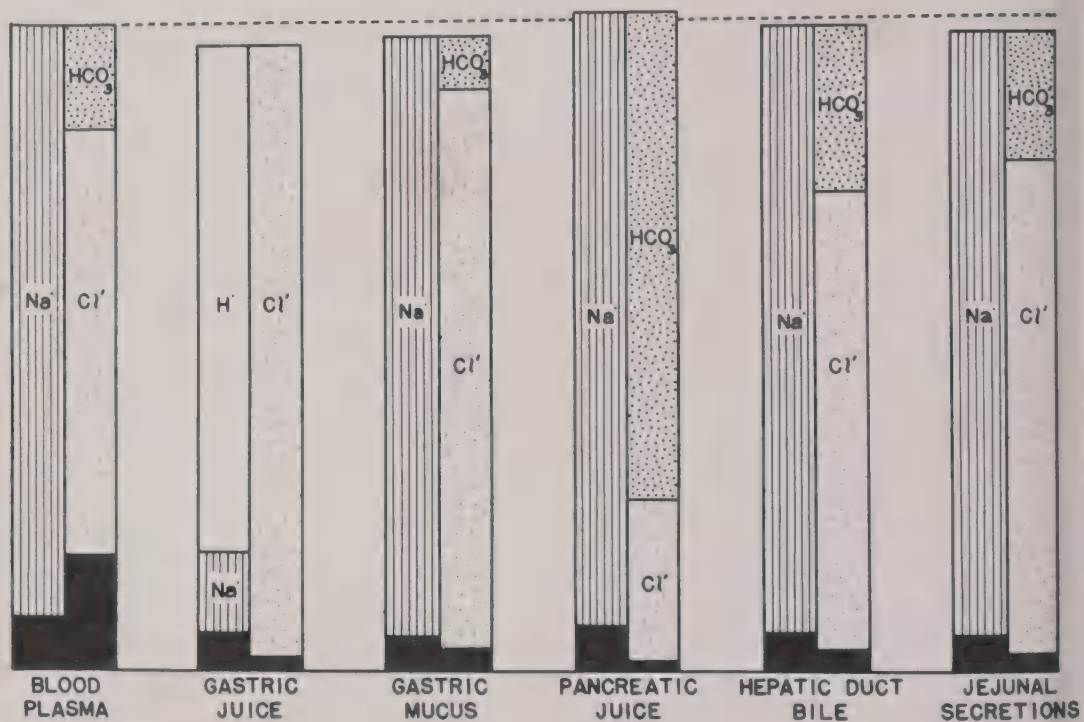


Fig. 67.—Electrolyte composition of gastrointestinal secretions. The  $K^+$  present in these secretions (included in the black block beneath the  $Na^+$ ) is usually 2 to 5 times that of plasma  $K^+$ . Gastric vomitus usually is a mixture of gastric juice and gastric mucus and the  $Na^+$  may be less than, equal to, or greater than the  $Cl^-$ . (From Gamble, J. L.: *Chemical Anatomy, Physiology and Pathology of Extracellular Fluid*, ed. 5, Cambridge, Mass., 1950, Harvard University Press.)

Similarly, in any condition involving an excessive loss of  $NaCl$  and water from the body, such as hemorrhage, or intestinal obstruction, potassium tends to leave the cells and go into the blood plasma. The potassium salts are excreted in the urine if renal function is satisfactory, but if this function stops then potassium accumulates in the plasma and toxicity is manifest. Potassium also leaves the muscles and other organs in certain disease states and after surgical trauma. Under these circumstances sodium frequently accumulates in excess of the potassium lost.

In untreated diabetes there is a loss of water, together with sodium and potassium, which are excreted in the urine as salts of the keto acids. When acidosis occurs, these losses are increased. Plasma  $HCO_3^-$  is diminished. Since

omiting frequently occurs, marked dehydration results from these losses of electrolytes and water, with effects pictured in Fig. 66. However, the administration of insulin and replacement solutions halts ketosis and further loss of water and of the electrolytes. (Atchley.) The acidoses of childhood and of starvation are also accompanied by similar losses of water and salts.

The adrenal cortex has a very profound influence upon electrolyte metabolism. More specifically it controls the level of sodium ions. In adrenalectomized animals there is a decreased concentration of sodium in the plasma and an increase in the potassium. Attention at first was centered on this rise in the potassium, but it now appears that the sodium is the more important factor. In Addison's disease, which is a condition involving adrenal insufficiency, the same relationships are seen. The low plasma sodium is a result of increased excretion of sodium by the kidneys. (Harrop.) It is lost not merely from the plasma, but also from the interstitial fluid, especially in the muscles, which at the same time gain water, in a manner analogous to the swelling of erythrocytes when placed in hypotonic saline solutions. (Muntwyler.) Loeb and his co-workers have found that treatment with sodium chloride will alleviate the symptoms of patients suffering from this disease. Administration of one of the adrenal cortical hormones, desoxycorticosterone or cortisone, in large amounts to animals with acute adrenal insufficiency results in an increased sodium concentration in the blood serum. This probably is due to a shift of interstitial fluid (tissue fluid) to the blood, resulting in a dilution of the blood with this fluid, which contains sodium salts. There then occurs a more rapid excretion of water and a diminished excretion of sodium salts, with a consequent improvement of the condition. In edema states, such as nephroses, cortisone frequently causes a diuresis of sodium and water.

The high potassium content of the blood in adrenal insufficiency seems to be due to a diminished ability of the kidney to excrete potassium. Along with an increase in the potassium content of the serum, there is also an augmentation of the potassium of the muscle cells. The large doses of adrenal cortical extract, which raise serum sodium in adrenal insufficiency, decrease serum potassium by enabling the kidney to excrete it.

In shock due to trauma or burns, there is no over-all loss of salt from the body, but there is internal loss and a marked change of the electrolyte pattern. The injured or burned tissues lose potassium, apparently by extrusion from the cells. Sodium passes into the cells in exchange for the potassium. These changes are proportional to the mass of damaged tissue. There is also a considerable gain of extracellular fluid (water and sodium) which is probably the source of the increment of intracellular sodium. The sodium present in the injured cells is really lost from the plasma and interstitial fluid and other injured functioning tissue. Tissues remote from the site of injury or burn do not show much change in water content but do show a loss of sodium and a gain of potassium, pointing to extracellular dehydration with intracellular swelling. (Fox and Baer.)



The loss of salts and water by sweating may be very considerable. When strenuous work is done, especially at high temperatures, as by miners or blast furnace workers, as much as from 10 to 15 liters may be lost in eight hours of work. If each liter contains 3 Gm. of NaCl, it can be seen that this represents a tremendous depletion of the salts of the interstitial fluid. When these stores are gone, first the plasma and then the cells suffer. Violent cramps ("stoker's" or "miner's" cramp) and prostration may result from the combined loss of salt and fluid. Replacement of the water alone may make matters worse by dilution of the plasma. To guard against this, the drinking water for such workers should contain 0.1-0.15 per cent of NaCl. This does not have an unpleasant flavor and allays thirst quite as well as unsalted water. In fevers, patients may lose large amounts of moisture and electrolytes in perspiration. These should be replaced if ill effects are to be prevented. An increased salt intake during hot weather has also been recommended for most people because of this loss of salt in the perspiration. In cases of renal insufficiency or edema more cautious replacement is necessary.

The simplest way to restore fluid in dehydration is to administer saline, usually 0.9 per cent NaCl. If there is acidosis, some alkaline salt may be used. Sodium lactate or sodium acetate, which are metabolically converted to bicarbonate, are generally preferred to sodium bicarbonate. (See Table XXXVII.) Glucose should not be administered unless there is ketosis because it tends to cause or increase diuresis and thereby accentuates the dehydration. Excellent results have been obtained by the oral administration of isotonic sodium lactate solution to restore fluid and salt balance in severe extensive third degree burns. (Fox.) In infantile diarrhea the addition of potassium to a mixture of sodium chloride and sodium lactate was found to improve the clinical results. (Darrow and Pratt.) When large volumes of fluid are administered in a variety of clinical conditions, the inclusion of potassium and other mineral supplementation is advisable to assist in obtaining normal extracellular fluid composition. (Fox, 1952.) The readily available solutions for intravenous therapy are compared in Table XXXVII. The frequent occurrence of multiple ionic alterations and the critical interrelationships among the various cations suggest the desirability of some type of "balanced electrolyte solution."

TABLE XXXVII  
COMPARISON OF PLASMA WITH REPLACEMENT SOLUTIONS\*  
(All concentrations in milliequivalents per liter)

SOLUTION	Na	Cl	HCO <sub>3</sub>	K	Ca	Mg
Plasma	140	103	27	5	5	3
Balanced electrolyte solution (Fox)	140	103	55†	10	5	3
0.9 per cent sodium chloride	154	154	0	0	0	0
M/6 sodium lactate	167	0	167‡	0	0	0
Ringer's solution, USP	147	155.5	0	4	4.5	0
Lactate Ringer's solution, <sup>1</sup> USP	130	109	28‡	4	3	0
Darrow's solution	122	104	53‡	35	0	0

\*From Fox, C. L., Jr., et al.: J. A. M. A. 148: 827, 1952.

†Obtained by metabolism of acetate 47 and citrate 8.

‡Obtained by the theoretical 100 per cent metabolism of DL-lactate.

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## Chapter 19

### URINE

The main function of the kidneys is the secretion of urine. They possess certain other functions also—the formation of ammonia to aid in the neutralization of acids and the secretion of some physiologically effective compounds. Primarily, however, the kidney is an excretory organ. The formation of urine from the glomerular filtrate was sketched in the preceding chapter. The urine thus formed carries off (1) water and salts in such amounts as will maintain the normal equilibria between the extracellular fluids and the intracellular fluids, (2) acids or bases to maintain a normal acid-base balance, (3) waste products, (4) toxic and detoxicated substances, and (5) other substances which are present in the blood in excessive amounts, if they can be so excreted.

#### GENERAL CHARACTERISTICS

Although it is frequently desirable to obtain and analyze “casual” specimens of urine, or the excretory output for short periods, it is usually customary to examine twenty-four hour specimens in order to have a “yardstick” for comparison and study. The physical characteristics which are usually noted in examining such a specimen are: volume, turbidity, color, odor, specific gravity, and reaction.

**Color.**—The color of normal urine is “amber yellow.” It is a color which is difficult to reproduce artificially in a fluid or to determine colorimetrically, because it results from a mixture of natural pigments and these are not always produced in the same proportion. The principal pigment is *urochrome*, which is yellow. This is a compound of urobilin and urobilinogen with a peptide, according to Drabkin. Small amounts of *uroerythrin*, and *uroporphyrin* are usually present. Uroerythrin, which is possibly derived from the melanins, is red, and uroporphyrin is a brownish-red iron-free pigment arising from heme metabolism. Riboflavin and one of its metabolic products uroflavin, may be present and give a yellow fluorescence to the urine.

On standing, urine usually deepens in color, a result of colorless “chromogens” changing to colored compounds. Thus, urobilinogen and urochromogen on oxidation yield urobilin and additional urochrome, respectively.

Abnormally, there may be excreted excessive amounts of some of the normal pigments, notably uroporphyrin. The color may also be changed by the appearance of hemoglobin, urobilin, bile pigments, or melanins. Foreign pigments, such as dyes, occasionally are found following their administration. Among the chromogens which are not normal is homogentisic acid. The latter occurs in that “inborn error of metabolism” known as alcaptonuria. In this condition the urine, when passed, is of normal color but assumes a “smoky” or blackish hue on standing. The darkening begins at the top, where it is exposed

to oxygen, and travels downward. Homogentisic acid is a product of the complete metabolism of tyrosine and phenylalanine, as shown previously.

**Volume.**—The rate of secretion of urine is not constant and depends upon a number of factors. Normally more urine is secreted during the day than at night, but this is reversed in the case of night workers. The food and fluid intake, the temperature and humidity of the atmosphere, and exercise are the chief influences. Young children excrete more urine in proportion to their weight than adults. Mental excitement also can influence the volume secreted. The total output in a twenty-four hour period in the northern part of the United States averages from 1,000 to 1,500 ml.; in the South it is likely to be somewhat less. This is, of course, related to the temperature. In summer, day's output may be as low as 600 ml. because of the diversion of water to the skin and lungs. Exercise results in a similar diminution, for the same reason. Loss of large quantities of water in diarrheal discharges will also lower the volume of urine.

Foods contain varying amounts of water, and some water arises in the oxidation of foodstuffs. Salty and spicy foods as a rule induce diuresis, and certain beverages, beer for example, have a decidedly diuretic influence. Among the diuretic drugs is caffeine and, therefore, coffee and tea have this property. Urea has the same effect; hence a high protein diet results in a larger output of urine.

Pathologically the volume of urine is increased as a result of injury to the posterior pituitary gland. "Diabetes insipidus" is a disease in which there is a deficient secretion of this gland. In this disease enormous volumes of urine are eliminated, with resulting intense thirst. An increased urinary output is designated "polyuria." Polyurias are also seen in diabetes mellitus, because glucose is a diuretic; in malnutrition; in certain endocrine imbalances; and in some renal conditions. In fact, an increased flow of urine at night is frequently one of the earliest symptoms of chronic kidney disease. This is called *nocturia* and is defined as the passage of a volume of over 500 ml. of urine having a specific gravity below 1.018 during a twelve-hour night period. This polyuria of the early stages of chronic glomerulonephritis is believed by some authorities to be an effort on the part of the kidney to compensate for the smaller number of healthy functioning renal units. Others, however, consider it to be a definite diminution of the ability of the tubules to reabsorb the water from the urine, which has filtered through the glomerulus, even if this be normal in quality and quantity. In later stages, because of the involvement of the glomerulus, urine volumes decrease. This is usually also the state of affairs in acute glomerulonephritis. A diminished secretion of urine is termed *oliguria* and a cessation is known as *anuria*. Oliguria also occurs in fevers, cardiac conditions, and in diarrhea. In fevers the explanation is that there is a shift in the water balance, much of the water of the blood going into the tissues temporarily. From a concentrated blood (anhydremia) the kidney cannot remove water. With a weakened heart action the kidney is not supplied with a sufficient quota of blood and therefore does not secrete urine efficiently. In diarrhea the loss of fluid results in anhydremia with consequent diminution in

the secretion of urine. Other causes of dehydration, such as persistent vomiting or excessive sweating, will similarly cause oliguria.

**Specific Gravity.**—The normal range of specific gravity is from 1.008 to 1.030, but usually it is within the limits of 1.015 to 1.025. In a general way it varies inversely with the volume secreted. In diabetes insipidus the specific gravity is very low, approaching 1.000, while in fevers, in which a small volume is secreted, the urine is concentrated and has a high specific gravity. An exception to the inverse ratio is diabetes mellitus. Here the volume is usually large and the specific gravity is high because of the glucose present.

Normally, urine secreted at night has a higher specific gravity than that secreted during the day. A considerable variation also is seen from hour to hour throughout the day. In fact, a constancy, or fixation, of the specific gravity over any appreciable length of time is considered abnormal and a sign of improper renal function. The principle involved is used in several concentration and dilution tests. In each of these, under a fixed set of conditions as regards the kind and amount of food and water taken, the urine is collected at specified intervals and the volume and specific gravity determined. In the concentration tests relatively dry food and little drink are given; a highly concentrated urine should be eliminated if the kidneys are normal. On the other hand normal kidneys are able to secrete a large volume of urine of extremely low specific gravity when a considerable quantity of water is drunk, as in the dilution tests. Abnormal kidneys cannot meet the same demands. Moreover, normally the specific gravity is not constant. For example, in the Mosenthal test, specimens are collected every two hours. The specific gravities of these samples will show a difference of at least ten points between the lowest and the highest in normal individuals. In Table XXXVIII are given some typical results. These tests are excellent criteria of the qualitative detection of renal dysfunction, but they do not give information regarding the extent of damage to the kidneys.

TABLE XXXVIII

TYPICAL TWO-HOUR SPECIFIC GRAVITY TESTS (MOSENTHAL)\*

TIME	NORMAL		EARLY NEPHRITIS		TERMINAL NEPHRITIS		MYOCARDIAL FAILURE	
	ML.	SP. GR.	ML.	SP. GR.	ML.	SP. GR.	ML.	SP. GR.
A.M. to 10 A.M.	150	1.015	75	1.016	140	1.005	65	1.024
A.M. to 12 Noon	155	1.020	70	1.017	160	1.004	80	1.022
to 2 P.M.	190	1.013	100	1.010	150	1.006	70	1.020
to 4 P.M.	250	1.010	130	1.009	180	1.004	85	1.022
to 6 P.M.	120	1.020	150	1.010	135	1.005	100	1.018
to 8 P.M.	245	1.011	180	1.009	115	1.005	50	1.024
Total for day	1,100		705		880		450	
P.M. to 8 A.M.	360	1.022	700	1.012	970	1.007	150	1.020
Total for twenty-four hours	1,470		1,405		1,850		600	

\*From Cantarow, A., and Trumper, M.: Clinical Biochemistry, ed. 2, Philadelphia, 1939, B. Saunders Co., p. 409.

The specific gravity affords a method of estimating the total solids excreted in the urine. Actual determination of total solids is time consuming and, be-





Hyaline Casts



Finely Granular Casts; also Pus Cast and Waxy Cast



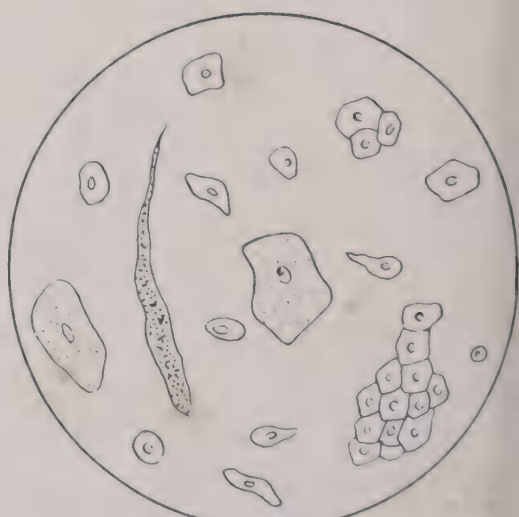
Coarsely Granular Casts



Pus Cells with Epithelial Cells and Stringy Mucus



Erythrocytes and Leucocytes



Various Types of Epithelial Cells; also Cylindroid



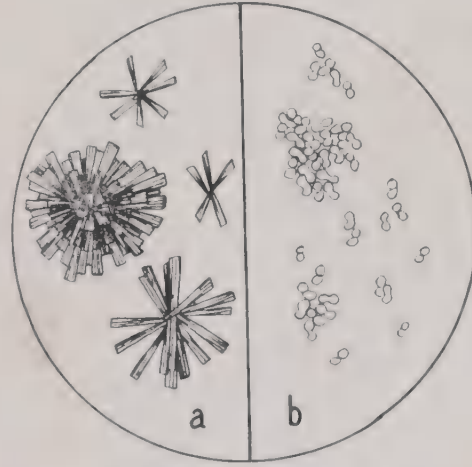
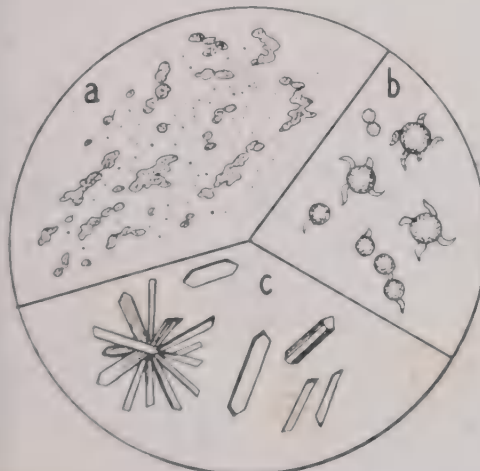
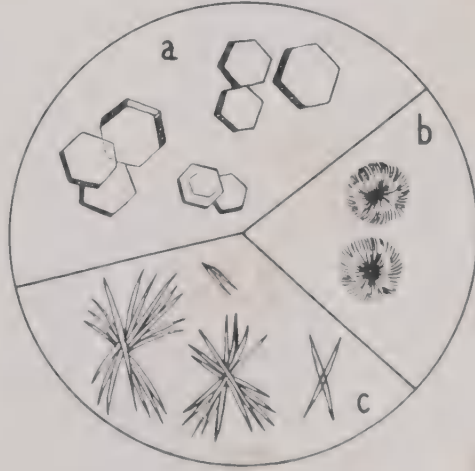
Ammonium Magnesium ("Triple") Phosphate



Calcium Oxalate



Uric Acid

a. Calcium Phosphate  
b. Calcium Carbonatea. Sodium Urate (Amorphous)  
b. Ammonium Urate  
c. Hippuric Acida. Cystine  
b. Leucine  
c. Tyrosine

cause of volatile substances present, not very accurate. Consequently a rough method based on a simple calculation is often used. The figure 2.6 (Long's coefficient) multiplied by the last two digits of the specific gravity at 25° C. is taken as the total solids in grams per 1,000 ml. of urine.

**Reaction.**—Normal human urine may be neutral, acid, or alkaline, having a pH range of from 4.8 to 7.5. It is usually acid with an average pH of 6. The reaction is dependent on the many different inorganic ions as well as organic compounds of acid and basic character present in urine.

Protein diets give rise, in general, to highly acidic urine. This is chiefly due to the sulfur of the sulfur-containing amino acids which is oxidized to sulfuric acid. The phosphoproteins in addition yield phosphoric acid, as do the nucleic acids and the phospholipids. Meats, therefore, are most productive of acid because of their high content of proteins, nucleic acids, and phospholipids. Alkaline urines are secreted when there is a predominance of vegetables and fruits in the diet, since in general they have an alkaline ash. (See page 323.) Thus the proportions of the various foods will influence the reaction of the urine. There is another factor which plays an important part. That is the production of ammonia by the kidney, and this modifies the amount of titratable acidity. The total titratable acidity usually is equivalent to 150 to 500 ml. N/10 acid per day.

Specimens of urine taken at intervals will usually vary a great deal in their acidity. Soon after meals the urine secreted is quite alkaline for a while. This "alkaline tide" is explained by the fact that hydrogen ions are secreted in great quantity in the gastric juice. This would result in an alkaline blood if the kidneys did not secrete a preponderance of base at that particular time.

Urine must not be permitted to decompose during or after the collection of a twenty-four hour sample. If microorganisms begin to grow they convert the urea to ammonium carbonate and the urine becomes "ammoniacal." Besides having an unpleasant odor, it indicates a change in the distribution of the nitrogenous constituents, with some loss of nitrogen as volatile ammonia. To avoid this, the urine may be kept cold, or a preservative, such as toluene, may be added to the container at the start of the collection period.

**Odor.**—Freshly voided urine, or urine which has not been permitted to spoil, has a not unpleasant odor, sometimes described as "aromatic." If urine does not have such an odor soon after it is passed, it may indicate some pathological state. A putrid or strongly ammoniacal smell would point to decomposition by bacteria, probably occurring in the urinary bladder. Other odors arise from foods eaten, such as the unpleasant one of methyl mercaptan after partaking of asparagus. Oil of sandalwood, cubebs, and other drugs give rise to characteristic odors in the urine. Methyl salicylate and oil of wintergreen give rise to a strong odor of wintergreen. Perhaps the most important odor, and one which sometimes aids in diagnosis, is the fruity aroma which is observed when a large amount of acetone is present.

**Turbidity.**—Normal urine is always perfectly clear and transparent when voided. On standing there is likely to separate out a faintly cloudy flocculence which is believed to be nucleoprotein or mucoid, present only in traces, together



with some epithelial cells. Turbidities may be of several types. Ammonium urate may precipitate from alkaline urine, whereas other urates are found only in acid urines. The former dissolve on acidification; the latter, on warming. Calcium phosphate and ammonio-magnesium-phosphate ("triple phosphate") are only seen in alkaline urines, or they may form a cloudy precipitate from an alkaline urine on warming. They dissolve on acidification. It is therefore quite essential to be sure that a urine sample is slightly acidified when making a test for heat-coagulable proteins. If a clear urine is acidified slightly without heating and a precipitate is formed, it is either a mucin or a nucleoprotein, since these proteins come down at an acid pH. A sediment in urine which does not dissolve on adding acid or on heating is most likely made up of cellular matter; i.e., pus, epithelial cells, or microorganisms.

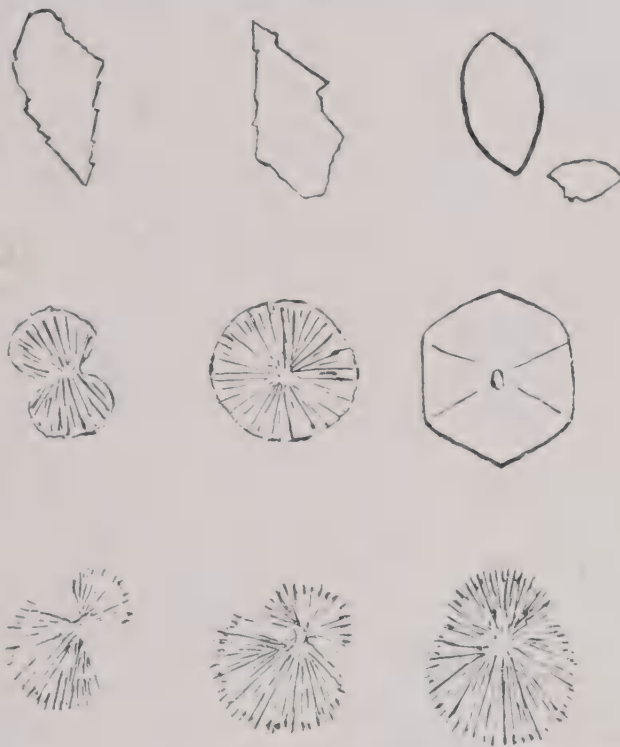


Fig. 70.—Crystals appearing in human urine after administration of (from top to bottom) sulfapyridine—arrowheads and whetstones; sulfathiazole—striated dumbbells (shocks of wheat with central binding), rosettes with radial striations, and regular hexagonal platelets (all structures *symmetrical*); sulfadiazine—striated dumbbells (shocks of wheat with excentric binding) and shell forms with radial striations (all structures *asymmetrical*). It is apparent that the sulfathiazole rosettes and the sulfadiazine shells grow out of their respective dumbbell forms. (Traced from microphotographs of urinary sediment  $\times 250$ .) (From Lehr, D., and Antopol, W.: Science 94: 282, 1941.)

A microscopic study of the sediment present in the urine is often of great assistance to the clinician in diagnosis. Both crystalline substances and cells afford many clues which an expert microscopist can utilize to advantage. A number of these cells and crystals sketched from typical fields is shown in Figs. 68 and 69. In Fig. 70 are shown crystals of some of the sulfa drugs likely to be found in the urine following therapy with these substances. A small number of leucocytes is always, or almost always, present. Only an increase is pathological, and it is then called "pus." Pus is usually accompanied by protein

and may arise from an inflammation of any part of the genitourinary tract. The presence of a small or moderate number of epithelial cells, also, is an ordinary occurrence, but a great number is abnormal. However, in the female, catheterized specimens are required for careful diagnostic work because fairly large numbers of red and white blood cells and vaginal epithelial cells are likely to be present in uncatheterized urine.

### GENERAL COMPOSITION OF URINE

Normal urine is composed of the following: (1) water, (2) inorganic salts, (3) nitrogenous organic compounds, (4) nonnitrogenous organic compounds.

The inorganic ions include the following:

#### Cations

Sodium, potassium, calcium, magnesium, ammonium; traces of iron, copper, zinc

#### Anions

Chloride, phosphate, sulfate, carbonate; traces of nitrate, silicate, fluoride.

There are also minute traces of many other inorganic compounds.

The nitrogenous organic compounds excreted in the urine are, with few exceptions, waste products. The most important ones are urea, uric acid, creatinine, creatine, hippuric acid, indican, purines other than uric acid, and amino acids.

Nonnitrogenous organic substances are less in amount and include traces of glucose, glucuronic acid, cholesterol, and the acetone bodies; oxalates and salts of other organic acids; and organic sulfur compounds.

**The Sulfur of Urine.**—Most of the sulfur of our diet is protein sulfur—in the amino acids cystine, cysteine, and methionine. Some enters the body also as chondroitin acid and there are small amounts in other forms. It is excreted as oxidized, or “acid sulfur,” i.e., as sulfate, or as “neutral sulfur.” Sulfates are both organic and inorganic. The former is called “ethereal” sulfate. There is no definite proportion among the three forms, the inorganic, ethereal, and neutral, but on an ordinary mixed diet from 79 to 84 per cent of the total sulfur is excreted in the form of inorganic sulfate. The remainder is divided between the ethereal sulfates and the neutral sulfur; about 4 to 7 per cent is ethereal sulfate and 16 to 21 per cent is neutral sulfur. (Freyberg.)

The sulfur of the amino acids is mostly oxidized to  $\text{H}_2\text{SO}_4$ , which combines with inorganic bases for the most part and, to a lesser extent, with organic compounds. These may be phenols, cresols, indoxyl, skatoxyl, or other compounds. Some of these are toxic and the formation of the “conjugated” sulfates transforms them into harmless products. The reactions are analogous to that shown for the formation of indican from indoxyl on page 521. Since the sulfates are derived from the sulfur-containing amino acids, the amount of total *sulfate* ex-

reted will, in a general way, be an index of the amount of protein metabolized, although not as accurately as the total nitrogen excretion. Hence, the total sulfate and the total nitrogen of the urine generally run parallel.

The remaining fraction of urinary sulfur, the neutral sulfur, includes a variety of compounds having the  $-SH$ ,  $-S-$ , and  $-SCN$  groups. It would, accordingly, include sulfur-containing amino acids, such as cystine and any peptides containing them. Also in this fraction would be thiosulfates, taurine, ergothioneine, urochrome, and the thiazole part of thiamine. The amount excreted is largely independent of protein intake, but is related to cellular protein metabolism.

Pathologically sulfate excretion is increased when tissue protein catabolism speeded up, as in acute fevers. Neutral sulfur excretion rises in cases of poisoning by cyanides and nitriles, because of the transformation of these compounds into thiocyanates and their excretion in that form. Chloroform and other anesthetics also increase the excretion of neutral sulfur. In cystinuria, naturally, this fraction is greatly increased.

**Phosphorus.**—The only phosphorus compounds found in urine in appreciable amounts are the derivatives of orthophosphoric acid. The total amount of phosphate eliminated in the urine will vary with the amount of phosphorus in the food and the amount absorbed. Food phosphorus is combined in the phosphoproteins, phospholipids, nucleoproteins, and preformed phosphate. If calcium or magnesium ions are present in the intestinal canal in abundance at the same time as phosphate ions, insoluble calcium or magnesium phosphate will be formed which will not be absorbed. Other insoluble phosphates are also possible, and consequently the urinary phosphate may represent only 50 to 70 per cent of the food phosphate. Most of the remainder goes through into the feces. The determination of the amount of phosphorus in urine is, therefore, of little value. There are certain major variations of the urinary phosphorus which are of interest, however. In acidosis the phosphoric acid or acid-phosphate excretion may rise (unless the kidney is incapable of secreting it, as may be the case in nephritis). This is a direct effort of the organism to get rid of hydrogen ions, preserve its base so far as possible, and maintain the normal pH. An increased elimination of phosphate is one of the first events in hyperparathyroidism, or after the administration of parathyroid hormone. Low urinary phosphate is likely to be associated with diarrhea, because the intestinal contents are hurried through the canal; with acute infections and nephritis, because of failure of the kidneys to function adequately; with pregnancy, as a result of the fetal requirement for phosphate; and with rickets and other bone diseases, in which there is a diminished absorption or increased intestinal elimination of phosphate. When insulin is administered there is an increased requirement of phosphate for the formation of hexose-phosphates. This results in a diminished urinary phosphate for a time, often followed by an increase.

**Chloride.**—About 10 to 15 Gm. of chloride, as NaCl, are ingested per day. Normally the amount eliminated in the urine is almost equal to that taken



in. Next to urea the chlorides of the urine are the chief solid constituents. Acid base balance and water balance are intimately associated with the distribution and elimination of NaCl. As has been seen, the sodium may be retained in time of stress, to conserve base. This is evident in the last stages of such a condition as pyloric obstruction.

It should also be remembered that as the filtrate, formed by the glomeruli of the kidney, is reabsorbed, much of the NaCl is absorbed. This is one of the "threshold substances" needed by the body and therefore retained in fairly definite concentrations. In Table XXXIX is shown a number of the common constituents of blood and urine with their relative concentrations. Sodium, calcium, and chloride ions have about the same concentrations in both urine and blood, which indicates that they are retained normally through the reabsorption mechanism of the kidney. In the case of sodium and chloride the evident result of this retention is a very large contribution to the osmotic pressure of the blood.

TABLE XXXIX  
RELATIVE CONCENTRATIONS OF CONSTITUENTS OF URINE AND BLOOD\*

SUBSTANCE	CONCENTRATION IN URINE (MG. PER CENT)	CONCENTRATION IN BLOOD (MG. PER CENT)	CONCENTRATION RATIO	CONCENTRATION IN BLOOD IN RENAL INSUFFICIENCY
Urea	2000	30	66.6	Increased
Uric acid	60	2	30	Increased
Creatinine	75	2	37.5	Increased
Indican	1	0.05	20	Increased
Phosphate	150	3	50	Increased
Sulfate	150	3	50	Increased
Potassium	150	20	7.5	Slightly increased
Chloride	500	350	1.4	Not increased
Sodium	350	335	1	Not increased
Calcium	15	10	1.5	Not increased
Water			1	Not increased

\*From Fishberg, A. M.: Hypertension and Nephritis, ed. 4, Philadelphia, 1939, Lea Febiger, p. 52.

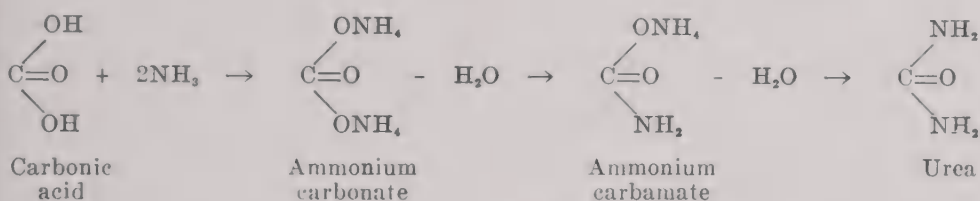
Deprivation of salt, as in salt-poor diets or in starvation, leads to a marked decrease in the volume of urine. It is the amount of chloride in the interstitial fluid which tends to determine chloride and water excretion. Ordinarily the addition of salt to a diet results in the elimination of the excess within forty eight hours. After a period of salt deprivation, such an excess will be retained until the volume and salt content of the interstitial fluid has been reconstituted.

An excessive loss of sodium chloride by way of the urine occurs in adrenal cortical insufficiency (Addison's disease). A clinical diagnostic test (Cutler's) is based on this fact. With a low sodium and high potassium diet for three days, the excretion of sodium by normal individuals averages 22 mg. per 10 ml. of urine on the third day, whereas that of patients having Addison's disease averages 206 mg. per 100 ml.

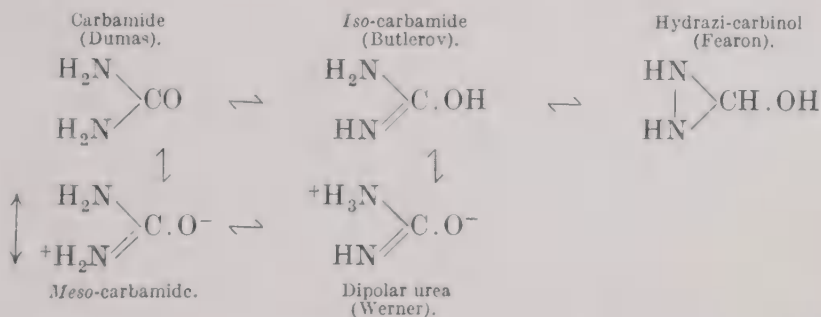
During the formation of the exudate in pneumonia, salt is removed from the other body fluids. Less salt is available, therefore, to the interstitial fluid, and both blood and urine chlorides drop. When the exudate is reabsorbed, the condition is reversed and larger quantities of chloride reappear in the urine.

**Positive Radicals.**—Ammonium, sodium, and potassium ions leave the body chiefly by way of the urine. Calcium and magnesium are excreted both through the intestinal tract and through the urine, chiefly perhaps by the former route. Ammonium salts will be considered among the nitrogen compounds.

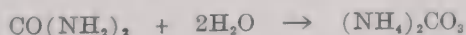
**Urea.**—Urea is the diamide of carbonic acid and, as such, is represented by the formula  $\text{CO}(\text{NH}_2)_2$ . This simple formula is in accordance with many of the reactions of this compound, including its preparation from ammonia and carbonic acid in vitro. Ammonia is caused to react with carbon dioxide, yielding ammonium carbamate. This is heated under pressure, liberating water and producing urea.



This formula, however, does not explain all the properties and reactions of urea, and a number of others have been suggested. Some of these are shown in the following diagram\*:



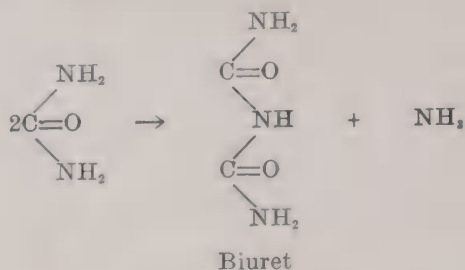
Urea is a white solid; it is odorless but has a bitter salty flavor. It crystallizes in long prisms. It is soluble in water and alcohol but not in ether or chloroform. The enzyme urease, which occurs in the jack bean and soybean, accelerates its conversion into ammonium carbonate.



This is the basis for the quantitative determination of urea in blood, urine, and other fluids. The ammonium carbonate formed is easily measured in a number of different ways.

\*From Fearon, W. R.: An Introduction to Biochemistry, ed. 2, St. Louis, 1940, The W. B. Saunders Co.

If dry urea is heated above its melting point, biuret is formed with the evolution of  $\text{NH}_3$ .



Biuret on treatment with cupric sulfate and an alkali yields a rose color. The well-known biuret reaction for peptides and proteins is based on this and indicates the presence of a grouping similar to the central portion of biuret. Although urea is neutral in reaction, it reacts with acids as a monobasic amide. Urea nitrate and oxalate form characteristic crystals insoluble in excess of the acid.

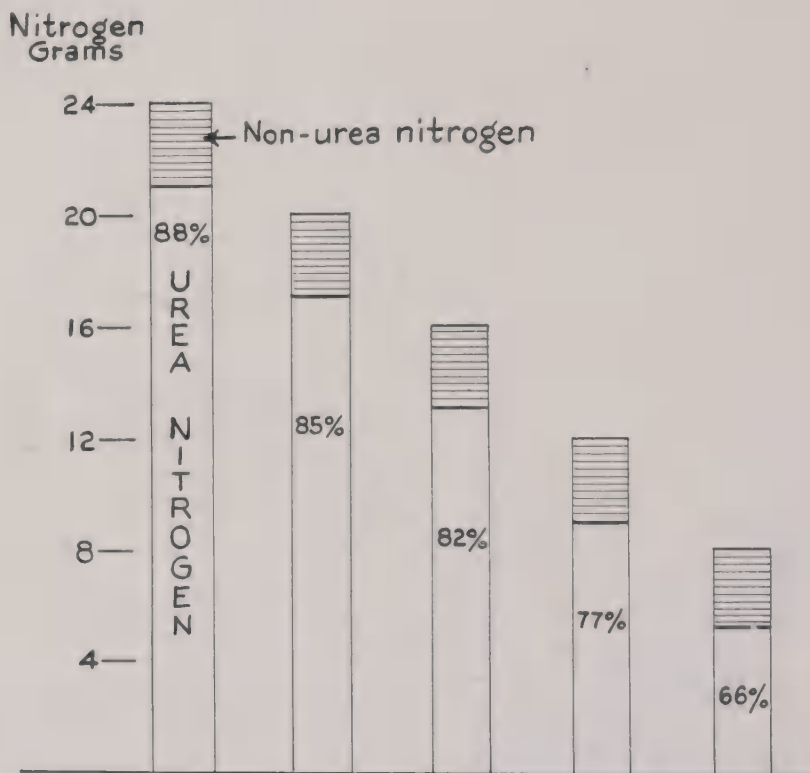


Fig. 71.—Relation of urea nitrogen to nonurea nitrogen at various nitrogen levels.

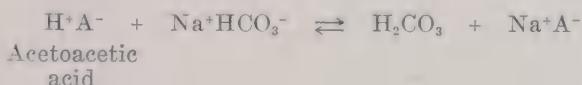
As has been seen, urea is the chief end product of protein metabolism. It is excreted in the urine in larger amounts than any other substance (about 30 Gm. a day) and makes up from 85 to 92 per cent of the total nitrogen on a medium or high protein diet. On a low protein diet the proportion (as well as the actual amount) of urea nitrogen is lower. It may be as low as 60 per cent of the total nitrogen. The reason for this is that the urea output parallels



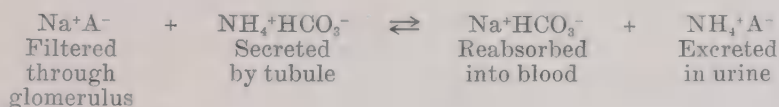
protein metabolism, while the nonurea fraction depends only in part on protein metabolism and in part on other factors. In Fig. 71 is indicated how, with a decreasing total nitrogen, the urea nitrogen assumes a smaller and smaller proportionate part of the total nitrogen.

It is nontoxic even when present in the blood in relatively large amounts. Consequently the reason high concentrations of urea are regarded with concern is not because of any inherent danger from the urea itself but rather because they indicate inadequate excretory function. Urea is a diuretic. It is not reabsorbed from the glomerular filtrate as it passes through the tubules and increases the osmotic pressure of the fluid.

**Ammonia.**—The total amount of urinary ammonia, as ammonium salts, is approximately 0.7 Gm. per day (0.5 to 0.8). It is formed in the kidney; its precursors have been discussed in Chapter 15. The production of ammonia aids in the neutralization of acids. By so doing it conserves bases like sodium and potassium which are essential for important physiological activities, such as buffer action. Thus when an acid like acetoacetic acid is produced in large amounts, it is thrown into the blood where it is buffered by  $\text{Na}^+\text{HCO}_3^-$ . Thus:



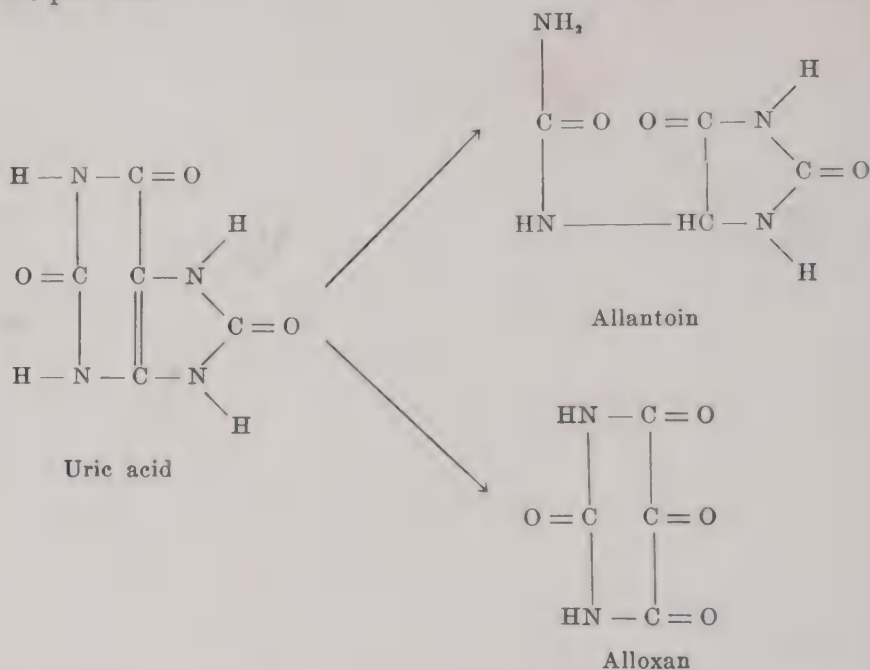
The  $\text{H}_2\text{CO}_3$  is excreted in the lungs. To restore the lost  $\text{NaHCO}_3$ , the following occurs in the kidney:



Normally the urinary ammonia comprises about 2.5 to 4.5 per cent of the total nitrogen. In acidosis this rises above 5 per cent, and at one time this was the method of determining the presence and degree of acidosis clinically. Since urea is easily decomposed and converted to ammonium carbonate, ammonia determinations must be made on fresh or sterile urine.

**Uric Acid.**—Acidified urine, on standing, will show a reddish crystalline deposit of uric acid. In fact, the normal acidity may be enough to permit this to take place. These crystals assume a variety of shapes—wedges, prisms, dumbbells, rosettes, etc. This impure uric acid may be purified by dissolving in concentrated sulfuric acid and pouring this into a very large volume of distilled water. Pure uric acid, or nearly pure, crystallizes out in white rhombic crystals under these conditions. This odorless, tasteless crystalline substance is insoluble in alcohol and ether, slightly soluble in boiling water, but soluble in alkalis and alkali carbonates. Its alkaline solutions have some reducing power for silver salts, copper salts, phosphomolybdates and phosphotungstates. The reaction of phosphotungstate is utilized in the Folin method for the determination of uric acid.

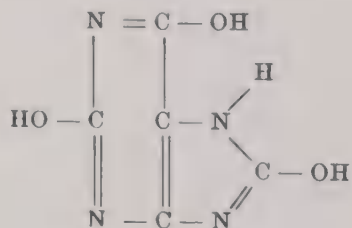
When oxidized in alkaline solution allantoin is formed, but in acid solution alloxan is the product.



Allantoin is an end product of purine metabolism in many animals but not in man. Alloxan is of interest as a substance which can produce diabetes experimentally. (See page 435.)

The murexide test is a color test for uric acid and other purines in a dry state. The dry material in a small porcelain evaporating dish is treated with a drop or two of concentrated nitric acid, and it is then dried on a boiling water bath. A bright red color is produced in the presence of uric acid, which changes to purple if a drop of ammonium hydroxide solution is added at one side and to violet if sodium hydroxide is touched to another portion. This is a useful test when analyzing urinary calculi.

Although uric acid has no carboxyl groups, it acts as a weak, dibasic acid. This is due to the enolization of the three OH groups as shown in the following formula:



This should be tribasic but apparently the third hydrogen is not dissociated. Thus we have salts of the type  $C_5H_3NaN_4O_3$  and  $C_5H_2Na_2N_4O_3$ . Of the alkaline salts, the ammonium salts are the least soluble; then, in increasing order of solubility, come sodium, potassium, and lithium. The fact that lithium urate

the most soluble gave rise to the use of "lithia waters" in the treatment of pathological conditions ascribed to an excess of uric acid, or uric acid deposits. The lithium was expected to expedite the elimination of uric acid as the more soluble lithium urate.

The total amount eliminated is approximately 0.7 Gm. per day. Its determination in urine, however, is of little clinical value. Since it is an end product of purine metabolism, it will of course fluctuate with the purine intake. Diets rich in nucleoproteins, such as meats, particularly glandular meats, meat extracts, and legumes, lead to an increased excretion of uric acid. Caffeine, theophylline, and theobromine are not converted into uric acid. However, on a purine-free diet, some uric acid is constantly excreted. This amounts to about 0.2 to 0.5 Gm. per day for an adult. This fraction is referred to as "endogenous" uric acid. If this is determined for a given individual, the excess above this figure, which he will excrete on a purine-containing diet, is termed "exogenous." Endogenous is supposed to refer to the metabolism of the body cells and exogenous to the metabolism of food. Although metabolism cannot be divided in such an arbitrary way, the terms are often useful and are frequently employed. The uric acid excreted on a purine-free diet must arise from synthesis. This has been discussed in Chapter 15.

The intensity of nuclear metabolism is frequently reflected in the uric acid output. Thus, in leucemia, in which there is a high degree of nuclear metabolism, the uric acid excretion is markedly increased. However, it must be remembered that nucleoproteins occur in the cytoplasm as well as in the nuclei.

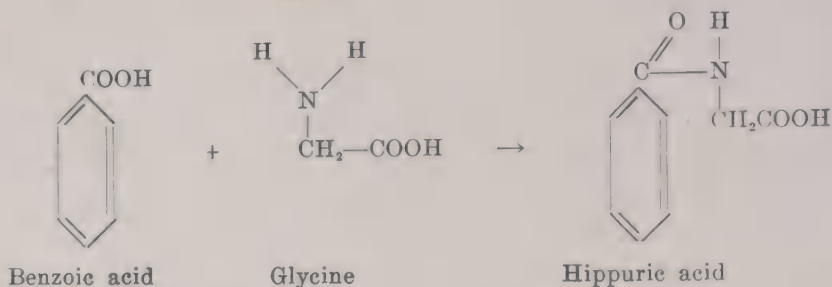
**Creatine and Creatinine.**—In Chapter 15 the relationship of these compounds to each other was discussed in detail. Creatinine is a constant normal constituent of urine, while creatine is an inconstant one. The former is easily determined by a colorimetric method, based on Jaffe's reaction. This is the production of a red-colored substance when a creatinine solution is treated with uric acid and alkali. Creatine is estimated, after transforming it to creatinine, by the same reaction. This gives creatine plus creatinine. The transformation is accomplished by long boiling in acid solution, or by heating in an autoclave at from 115 to 120° C. for twenty minutes.

Creatinine also yields a deep red color when a few drops of fresh sodium ferric prusside solution are added and the fluid is made alkaline. This color disappears after acidification with acetic acid. The importance of this test lies in the fact that acetone, a pathological constituent, gives a similar color under the same conditions, but it is not dispelled by acid.

The amount of creatinine eliminated varies chiefly with the weight of the individual, unless he is obese. That is, it has some relation to the muscular mass. The "creatinine coefficient" for men is about 18 to 32 mg. per kilogram body weight, and for women, 9 to 26 mg. per kilogram. A round number is total of about 1.5 Gm. per day.



**Hippuric Acid.**—Hippuric acid is so-called because it was first found in the urine of horses. It is benzoyl glycine, a compound of benzoic acid and glycine.

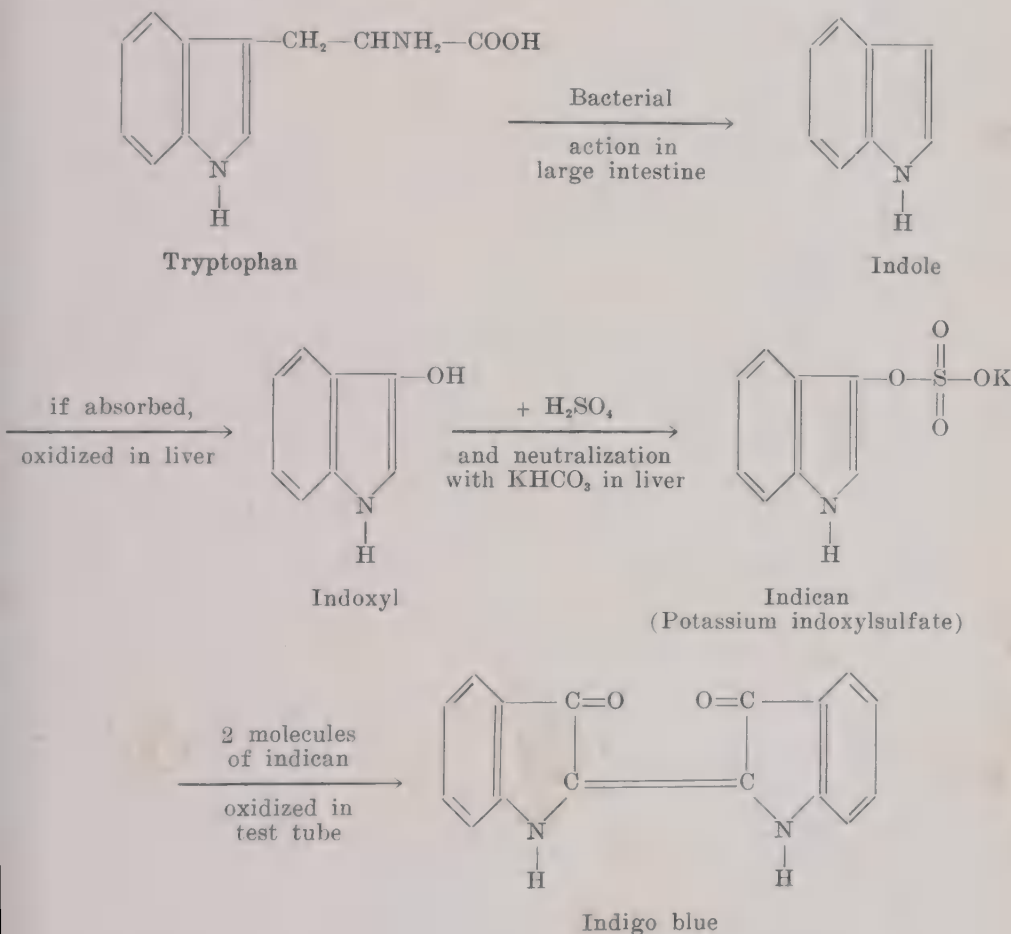


This is a physiological metabolic reaction which results in the “detoxication” of benzoic acid and benzoates. These occur in vegetable foods or are derived from the oxidation of aromatic substances. About 0.7 Gm. per day is eliminated on the average diet, but deviations from this are to be expected, depending upon the amount of the precursors in the diet. Glycine is usually present in sufficient amounts to combine with any quantity of benzoates which are likely to be ingested. Cranberries contain from 0.01 to 0.09 per cent of benzoic acid, and other fruits and berries smaller amounts. Some foods such as ketchups are permitted by law to have 0.1 per cent sodium benzoate added as a preservative. This is, of course, also converted into hippuric acid. Hippuric acid can easily be isolated from urine containing considerable amounts of it by concentrating and then acidifying. Both uric acid and hippuric acid will crystallize out, but they can be separated by the use of hot water in which hippuric acid is more soluble than uric acid. After again concentrating and acidifying, the hippuric acid will crystallize out in long, rhombic prisms.

In man the synthesis of hippuric acid takes place almost entirely in the liver. It is accomplished by the action of hippuricase, an amidase which also hydrolyzes hippuric acid to its constituents. Both ATP and coenzyme A are required for this biosynthesis (Chantrenne). This synthetic action has been used as a test for liver function, but it is evident that it is only a test for this particular liver function—not for all (Quick). Nevertheless it is claimed that subnormal values have been obtained in patients with various types of hepatitis and other liver conditions but not in those with uncomplicated obstruction of the common bile duct; that is, it may aid in differentiating between hepatic and obstructive jaundice.

The test consists in administering by mouth 5.9 Gm. of sodium benzoate in 30 ml. of water one hour after a breakfast of toast and coffee. One-half glass of water is then taken and the bladder is emptied, and the urine is collected hourly for four hours. Although the hippuric acid may be determined in each specimen, the total amount excreted is the most significant fact. During the four hours a normal person excretes about 3 to 3.5 Gm. of hippuric acid while a poorly functioning liver will not form that amount. There are several methods of determination, the simplest one being to acidify with HCl, filter off the hippuric acid and dry and weigh it, or it may be estimated by titration with standard alkali. A correction must be made to account for the amount of hippuric acid which remains in solution

**Indican.\***—Indican is the salt or salts of indoxyl sulfuric acid. It is derived from indole, which in turn arises from the action of putrefying bacteria upon tryptophan or of proteins containing it. This occurs in the large intestine. Any indole is absorbed, it undergoes a series of "detoxication" transformations, probably in the liver, and indoxyl is formed. This is conjugated with sulfuric acid and neutralized to yield a salt. Indican is detected in the urine by oxidizing it to indigo blue. Obermayer's reagent, which is concentrated hydrochloric acid containing a small amount of ferric chloride, is a good oxidizer for this purpose. Chloroform is then added and, after shaking gently, the blue dye is taken up by the chloroform. The formation of indican and its oxidation to indigo blue are shown below.



The steps between tryptophan and indole are not known, but it is thought that they occur in the manner shown on page 250. It should be remembered that indole is not formed in the normal metabolism of tryptophan in the body. Indican is a product of putrefaction, usually in the intestine, but possibly in other locations. Normally, according to Hawk and associates, from 4 to 20 mg. are ex-

\*The term "indican" is employed here in the usual clinical sense. This is not accurate from the organic chemical standpoint, according to which indican is a colorless glucoside, a combination of indoxyl with glucose, found in the indigo plant. This is hydrolyzed by enzymes to glucose and indoxyl. The indoxyl is then oxidized by the air to indigo. The clinical "indican" is more properly designated "indoxyl sulfate," but long usage warrants the term "indican."

creted daily, but a qualitative reaction may not be positive for this amount. An increase in urinary indican is found when there is increased putrefaction, provided the products are absorbed. (Putrefaction, it will be remembered, is the anaerobic bacterial decomposition of proteins.) Among the pathological conditions in which this is likely to be observed are hypochlorhydria, because of diminished bactericidal action of the gastric juice; intestinal obstruction and paralytic ileus, because the peristaltic movement is inhibited; and obstructive jaundice, because the absence of bile produces voluminous feces with higher nutritive value for the bacteria. Indicanuria is rather rare in simple constipation and rather common in diarrheas. Furthermore, some individuals showing no gastrointestinal symptoms excrete large amounts of indican continually, while others, with and without such symptoms, have no indican in their urine. It is, thus, an index of absorption of putrefactive products but does not necessarily have any other significance.

If putrefaction occurs elsewhere than in the intestinal canal, indole is produced and absorbed and follows the same course. The bacterial decomposition of tissue proteins or of the proteins of body fluids, such as exudates, occurs in gangrene, abscesses, empyema, etc., and may lead to a marked indicanuria.

**Non-nitrogenous Organic Compounds.**—Very small but variable amounts of a number of other organic compounds are found in the urine. The range of glucose in the urine of normal individuals is from 0.01 to 0.10 per cent, but usually it is in the lower part of that range, and a negative Benedict's test is almost invariably observed in normal urines. Glucuronates are formed after the administration of camphor, chloral, menthol, phenol, morphine, aspirin and other drugs. Some of these, it will be remembered, also combine with sulfuric acid, forming ethereal sulfates. The "conjugated" glucuronates will reduce alkaline copper solutions and rotate the plane of polarized light to the left but are not fermented by yeast. The sex hormones are excreted as conjugated glucuronates; they are more soluble in water than the uncombined hormones.

The "ketone bodies," or "acetone bodies," are present in normal urine in very small amounts, less than 0.1 Gm. in twenty-four hours. They consist of beta-hydroxybutyric acid, acetoacetic acid, and acetone. These substances arise in normal fatty acid catabolism in the liver and are carried by the blood to the extrahepatic tissues where their further degradation occurs. The concentration in the blood is usually less than 1 mg. per 100 ml.

Oxalates are probably excreted constantly in minute amounts. If present in sufficient concentration, they will unite with calcium to form insoluble calcium oxalate. This forms characteristic crystals of two types: dumbbell and octahedral. Citrate also is a normal constituent of urine and is assuming more importance continually. About 0.2-1.2 Gm. is found in adult urine in the twenty-four-hour output under ordinary circumstances. It is increased following the administration of alkali or during a high carbohydrate and fatty dietary regime. This "extra" citrate does not come from the citrate present in the bones but arises metabolically. The output of citrate also is related to changes in the steroidal reproductive hormones, but this may be an indirect



effect. The significance of these facts is not apparent. (Smith and Meyer; Huck; Shorr.) Undoubtedly other organic acids occur in urine in small amounts but, because they do not crystallize out or give characteristic reactions, they have not been studied extensively.

**Vitamins, Hormones, and Enzymes.**—The water-soluble vitamins are excreted by the kidneys in variable amounts. If excessive quantities are present in the diet, some, but not necessarily all, of the excess is eliminated. For some of these vitamins the determination in urine has been used as a measure of efficiency. For example, the excretion of thiamine on an adequate diet amounts to 90  $\mu$ g or more in males and 60 or more in females. On a deficient diet it is 5 and 43, or less, respectively (Robinson). Sometimes "saturation" experiments have proved valuable, that is, the response to the administration of a large dose of the vitamin. The excess eliminated reflects the degree of saturation previously present. However, the excess is not always eliminated quantitatively. Niacin amide and its derivatives are found in the urine normally to the extent of from 1 to 2 mg. together with 5 to 15 mg. of the physiologically inert trigonelline, a methylated derivative. Only 27 to 42 per cent of administered niacin amide is eliminated in urine (Sarett). On a normal average diet, there is ordinarily excreted from 15 to 28 mg. of ascorbic acid in twenty-four hours (Hawley). In an avitaminosis, as would be expected, the excretion of the particular vitamin involved is diminished.

As mentioned previously, the sex hormones are secreted in the urine conjugated with glucuronic acid. A great deal of research has been done regarding the variation in the excretion of the individual ones normally and under various conditions, but a consideration of this would be beyond the scope of this work. The urine of pregnant women contains a hormone, sometimes called the "anterior-pituitary-like" substance (A. P. L.). This is secreted by the chorionic cells of the placenta. Urine containing this hormone, when injected into an immature female mouse, causes marked changes in the ovaries, and the procedure, known as the Aschheim-Zondek test, is used as a diagnostic test for pregnancy. In modification, the Friedman test, employing adult female rabbits, is now more generally used.

The enzymes present in urine are small in amount and of little significance. It is unlikely that the enzymes of the blood are excreted in appreciable quantities. Those found in urine probably arise from the disintegration of leucocytes and epithelial cells which always occur in urine. An exception is diastase, which is found in the urine in fairly high concentration in acute pancreatitis. Another exception is urinary pepsinogen, which seems to vary directly with the excretion of gastric pepsin. (Janowitz and Hollander.)

## PATHOLOGICAL CONSTITUENTS

**Glucose.**—The term *glycosuria* is usually used for "glucose in the urine," though it really should mean "sugar in the urine," and glucosuria is a more accurate word in this connection. The other kinds of glycosuria include pentosuria, lactosuria, galactosuria, and fructosuria.

More than a trace of glucose in a twenty-four hour specimen of urine is pathological. Easily detectable amounts may be found in specimens voided soon after a high carbohydrate intake, but this "alimentary glycosuria" seldom gives a positive Benedict's test when the entire day's output is pooled and analyzed. In diabetes mellitus the urine is usually light colored, with a higher specific gravity than the color would seem to warrant and a glucose content of from a few tenths of a per cent up to 12 or 15 per cent. The severity of the condition cannot be gauged by the percentage alone, since this can be modified by varying the volume of fluid ingested. It is the actual number of grams excreted in twenty-four hours which is important. If the dietary carbohydrate and potential carbohydrate is calculated and the amount of glucose excreted is subtracted from it, the remainder will be the number of grams of glucose utilized. This gives the physician a basis for determining what diet to prescribe and whether or not insulin is necessary. The blood sugar must also be determined and taken into account; this will be discussed later. Suffice it to say here that if there is a normal renal threshold, that is, if the blood sugar does not rise above about 160 mg. per 100 ml. before glucosuria results, the urinary output is a very good guide for treatment. In certain renal conditions—glomerulonephritis, nephrosclerosis, and nephrosis—glucosuria may occur. This seems to result from a lowered renal threshold and is thus probably not due to any derangement in carbohydrate metabolism.

Besides diabetes mellitus, glucosuria, as a result of high blood sugar, accompanies about a fourth to a third of cases of hyperthyroidism. Hyperpituitarism and hyperadrenalism belong in the same category. Ether anesthesia, asphyxia, acidosis, and a variety of other conditions also lead to hyperglycemia and glucosuria. The explanation in each case, so far as it is known, is given in Chapter 22, in the discussion of hyperglycemias.

**Lactose.**—Lactose may be found in the urine in a considerable proportion of lactating women. According to Trumper and Cantarow, lactosuria seldom occurs *during* normal pregnancy. However, glucosuria is present in from 10 to 15 per cent of all normal pregnant women, with no accompanying hyperglycemia. Since it usually disappears later, it is frequently assumed to be lactosuria. No such assumption is justified, and the urine should be analyzed carefully to determine what sugar is present. Sometimes an early diabetes is not diagnosed because no differential analysis or blood sugar determination is made. Lactose may easily be distinguished from glucose in that it is not fermented by baker's yeast, gives a positive mucic acid test, yields lactosazone, and reacts negatively with the modified Barfoed's reagent. The amount of lactose eliminated is usually small.

**Pentose.**—An alimentary pentosuria, as a result of ingesting large amounts of prunes, plums, cherries, grapes, or their juices, is likely to be noted in normal individuals. Like alimentary glucosuria, it is temporary and has no significance. The excretion of a pentose in the urine has been reported in cases of morphine addiction. However, the most interesting type of pentosuria is the chronic type. This is an "inborn error of metabolism." The individual is born with the condition and no cure is known for it. However, the utilization of other carbohy-



rates is not impaired, and the only danger to the person having this derangement is that it might be mistaken for diabetes mellitus.

The urinary pentose, L-xyloketose, may be detected in urine by several methods. A rapid procedure is to add 0.5 ml. of benzidine in glacial acetic acid (1:25) to 0.1 ml. of urine. After heating to boiling, the test tube is cooled and 1 ml. of distilled water added. A rose-pink color is positive for pentose (Tauber). Lasker and Enklewitz have shown that L-xyloketose reduces Benedict's qualitative reagent more rapidly than does glucose. If 1 ml. of urine and 1 ml. of the reagent are mixed in a test tube and placed in a bath at 55° C., a yellow precipitate will appear in ten minutes in the presence of 0.1 per cent or more of the pentose. Fructose gives the same reaction since it also is a pentose. These two can be easily distinguished from each other because fructose is fermented by yeast and forms a glucosazone with phenylhydrazine.

**Other Sugars.**—Galactosuria has been observed in nursing infants suffering from gastrointestinal disturbance. It is of little importance. Fructosuria is said to occur occasionally in association with glucose, in severe cases of diabetes mellitus. There is also a rare condition, known as "essential fructosuria" or "levulosuria," in which no other carbohydrate is involved. It may be regarded as another "inborn error of metabolism," because individuals suffering from it have it from birth (Silver and Reiner). Insulin does not help the patient to utilize fructose. The site of the difficulty is believed to be the liver where fructose normally is stored as glycogen. Possibly there is a deficiency of a specific enzyme needed for this conversion. No other symptoms are peculiar to this condition, which does not lead to diabetes mellitus or to any change in the utilization of other carbohydrates. The method of detecting fructose was mentioned incidentally under pentosuria. Ribosuria has been observed in patients with progressive muscular dystrophies, myotonia congenita, and amyotonia congenita, but not with myasthenia gravis or progressive neuropathic atrophy. A provisional test for ribose is a positive Benedict qualitative test (in the absence of other sugars) after forty-five minutes' heating (Orr and Minot). A seven-carbon sugar, D-mannoheptulose, appears in the urine of normal individuals after eating large amounts of avocado. Although some of this sugar is utilized, enough is excreted to be a possible source of confusion in diagnosis.

**Fat.**—Alimentary "lipuria" may be observed when a large amount of fat has been ingested. Cod-liver oil in great quantity is an example. The urine is opalescent, or turbid, or even milky when voided. After standing, a peculiar creamy layer is seen at the top in those rare instances in which the fat content of the urine is high. The high blood fat (lipemia) which sometimes occurs in diabetes mellitus and lipid nephrosis may lead to lipuria. The same results may be observed following fractures of the long bones with injury to the bone marrow, which is rich in fat, and any injuries to the subcutaneous layer of fat. Other conditions in which fatty urines may be seen are pyelitis, pyonephrosis, lipid nephrosis, and in alcohol or phosphorus poisoning.

"Chyluria" is the term applied to the condition resulting from an obstruction to the thoracic duct. This is even more infrequent than lipuria. The



lymph vessels of the urinary tract become distended and burst, allowing lymph to pass directly into the urine. The appearance of the urine in chyluria is milky rather than opalescent.

**Proteins.**—The amount of protein which is excreted in normal urine is insignificant. It probably consists of serum albumin and serum globulin from the blood, and nucleoprotein or mucin, or both, from the epithelial cells of the urinary tract. The amounts of the latter two are so small that their identity is in doubt. Normal urine does *not* give positive reactions with any of the ordinary protein tests.

Abnormally, proteins appear in urine in varying amounts. The condition is commonly known as “albuminuria,” although the albumins seldom are found alone, and consequently the term “proteinuria,” which is being more and more generally employed, is to be preferred. The proteins, which are found in the urine in kidney conditions, are commonly believed to be plasma proteins which pass the damaged renal epithelium. The albumins, with the smallest molecules, pass most easily, globulins next, and fibrinogen least readily. However, some of the authorities are of the opinion that the plasma proteins undergo some slight change and, therefore, are, in a sense, foreign proteins. Since foreign proteins are promptly eliminated by the kidney, these are eliminated in the same way.

Proteinurias may be grouped in two general classes: Functional and organic. Functional proteinurias are those which are not related to a diseased organ. The amount of protein excreted is usually small, and the condition is ordinarily temporary. Violent exercise is an example. Soldiers, after long marches, and athletes, after strenuous contests, frequently have proteinuria. Here there may be a slight kidney damage to account for it, but the condition almost always clears up. Cold bathing, leading to constriction of renal blood vessels and anoxia, is another cause, and occasionally an alimentary proteinuria occurs after excessive protein ingestion. In all of these, the subjects may be of any age, but usually they are children or adolescents. This is especially true of orthostatic, or postural proteinuria and similar states. In these young people, usually 14 to 18 years of age, the urine contains protein when they are in the upright position only. When lying down it is free from protein. This is not an evidence of kidney disease but is probably due to some disturbance in the blood supply to the kidneys, leading to venous stasis and consequent anoxia. These benign proteinurias usually disappear within a few years, but sometimes they continue into adult life. Proteinuria is frequently associated with pregnancy, probably as a result of pressure interfering with the return of blood in the renal veins.

There are many pathologic conditions which cause “organic” proteinuria. These may be classified conveniently as (a) prerenal, (b) postrenal, and (c) renal.

The prerenal conditions which cause proteinuria are those which are primarily not related to the kidney. In most cases, however, they affect the kidney in such a way as to render it more permeable to the protein molecule. For example, cardiac disease, by affecting the circulation of the kidney, leads to pro-

teinuria. Any abdominal tumor, or mass of fluid in the abdomen, will do the same by exerting pressure upon the renal veins. Fevers, convulsions, anemias and other blood diseases, liver diseases, and many other pathologic states belong in this category.

Postrenal proteinurias are sometimes called "false" proteinurias, all others being "true." This is because they are those conditions in which the protein does not pass through the kidneys. It may be some inflammatory, degenerative, or traumatic lesion of the pelvis of the kidney, the ureter, bladder, prostate, or urethra. Bleeding into this tract will, of course, contribute proteins. Urine containing pus will also contain protein, since the exudate which accompanies it is rich in protein.

Proteinuria accompanies various types of kidney diseases. These are the "renal" proteinurias. In acute glomerulonephritis, protein is always found in the urine. In the chronic form of this disease proteinuria is seen in the early stages but may disappear later as the kidney becomes more and more impaired. In nephrosclerosis, albuminuria is frequently, but not always, found, and the same is true of tuberculosis and carcinoma of the kidney. There are several types of nephrosis—conditions characterized by degenerative lesions of the renal parenchyma. Protein is almost always excreted in nephroses, varying from small to large amounts. Lipoid nephrosis is a particular form of chronic kidney disease in which lipid deposits occur in the tubules. It is, however, considered to be an affection of the glomerulus. In this disease, large quantities of albumin are lost in the urine, and since this is derived from the blood, the concentrations of the blood proteins, particularly serum albumin, fall considerably. A number of investigators believe that lipoid nephrosis is merely a modified glomerulonephritis—not an entirely different condition. This view is supported by evidence that in some cases of definite chronic nephritis the character and quantity of proteins in the urine are the same as those found in the urine of patients with nephrosis (Blackman).

Polypeptides, the so-called "proteoses" and "peptones," sometimes are excreted in the urine. This may happen in pneumonia, diphtheria, carcinoma, and other conditions. This is because some protein-containing material, such as an exudate, or a tissue mass, or pus, is undergoing "autolysis"; i.e., self-digestion. If any soluble products of proteolysis, which have molecules too large for direct utilization or deamination, get into the circulation, they are excreted as foreign bodies.

A peculiar protein is eliminated by some patients having multiple myeloma, tumorlike hyperplasia of the bone marrow, and also in some other diseases of the bone marrow, and sometimes in leucemia. This is the Bence-Jones protein. It precipitates on warming the urine to 40 to 60° C., but dissolves almost completely when the temperature is raised to 100° C. Upon cooling, the protein precipitate reappears. It is believed to be a globulin of comparatively low molecular weight; i.e., about 37,000. An increased protein diet does not seem to be followed by a greater output of Bence-Jones protein.

Nucleoproteins are found in the urine in inflammation of the urinary epithelia, such as pyelitis, cystitis, and even in nephritis, at times. Since these



proteins precipitate in the cold on addition of mineral acids, they have frequently been designated mucins, which have the same property. Mucins probably also occur in urine, but not as frequently as the nucleoproteins.

**Hematuria** is the occurrence of blood in urine. This means whole blood, including erythrocytes, and is a result of hemorrhage. **Hemoglobinuria** refers to the excretion of hemoglobin and follows a hemoglobinemia. Excessive hemolysis precedes hemoglobinemia; that is, the red cells are laked by some hemolytic agent. Apparently hemoglobin, when it is in solution in the blood plasma, is treated by the body as a foreign substance and is excreted into the urine. However, there is a renal threshold for hemoglobin. If the kidneys are normal this is at a concentration of about 155 mg. per 100 ml. of blood plasma, but it may be lower when the kidneys are damaged. When the threshold is exceeded and hemoglobin is excreted into the urine, it may precipitate in the tubules if the urine is acid in reaction. Hence, in the treatment of hemoglobinuria, as, for example, following incompatible blood transfusions, the administration of alkalis may prove to be helpful. In attempting to differentiate between hematuria and hemoglobinuria, it should be noted that the red cells may disintegrate, especially if the reaction is alkaline, and the hemoglobin dissolve out.

**Amino Acids.**—Small amounts of amino acids, both free and combined, are excreted in the urine. Thompson and Abdunabi found that normal adult women excrete more amino acids than men. The free amino acid amounts to about 1.4 mg. per kilogram for men, and 2.3 mg. per kilogram for women, and there is some evidence for the existence of individual patterns of amino acid excretion. That is a given person will tend to excrete more of one amino acid and less of another than a second person, et cetera. According to Childs, this sex difference does not hold for children, who excrete somewhat less than adults; infants and prematurely born babies excrete about four times as much per kilogram as do older children. Increased excretion of amino acids may occur pathologically. It is frequently observed in diseases affecting the parenchyma of the liver, presumably because of the inability of the liver to deaminate the amino acids. Thus, in acute yellow atrophy of the liver the amino nitrogen level may rise to 40 mg. per 100 ml. However, even greater amounts have been found in the urine of a patient having no *obvious* liver damage. This was a case of hepatolenticular degeneration, a disease in which there is cerebral degeneration accompanied, or followed, by cirrhosis of the liver. Alanine, glutamic acid, and aspartic acid were identified (Uzman and Denny-Brown). The occurrence of cystine in the urine is discussed on pages 384 and 531.

**Ketone Bodies.**—The “ketone bodies” are acetoacetic acid, beta-hydroxybutyric acid, and acetone. It has been seen (Chapter 17) that the first two are normal products of fatty acid disintegration. They are formed in the liver, and destroyed or utilized by the extrahepatic tissues. If these two activities balance, there will be no excess in the blood; i.e., no “ketonemia.” If the formation by the liver is too rapid for the extrahepatic tissues to keep pace with it, ketonemia will result, and ketonemia is followed by ketonuria.



Acetone is generally believed to be a secondary product; that is, it results from the decomposition of acetoacetic acid.



Ordinarily a normal person on a mixed diet will excrete less than 0.1 Gm. of ketone bodies in twenty-four hours. In "ketosis," as the condition of excessive ketone production is termed, values as high as 100 Gm. per day, or even higher, have been reported. Ketonuria may be expected to occur in the acidosis of diabetes mellitus, starvation, in normal and toxic pregnancies, after ether anesthesia, and often in alkalosis. It is therefore not necessarily a sign of acidosis, although the most severe ketoses, with marked ketonuria, are seen in severe cases of diabetes mellitus. Acetone and acetoacetic acid are easily detected by qualitative color tests. However, the common Gerhardt test for acetoacetic acid, which is the appearance of a bordeaux red color upon the addition of ferric chloride, may be masked if salicylates or certain other compounds are present. If this is suspected, a portion of the urine may be boiled and then tested. A positive test before boiling, followed by a negative one after boiling, is definite evidence that the color was caused by acetoacetic acid. However, a positive test before and after boiling leaves one in doubt as to whether acetoacetic acid is present or not in addition to the disturbing drug. In such an event the acetoacetic acid may be extracted from the urine and tested separately. Beta-hydroxybutyric acid can best be detected by polariscopic examination. If glucose is also present, it must first be removed by fermentation. After clarification the fermented urine is examined in the polariscope, and if levorotation is observed, the presence of beta-hydroxybutyric acid is indicated. Glycuronates are also levorotatory, but they have reducing power, whereas beta-hydroxybutyric acid does not.

**Bile and Derivatives.**—Both bile pigments, or their derivatives, and bile salts may be found in urine in pathologic states. If there is a stasis, or damming back of bile, it will of necessity get into the blood and be excreted in the urine. Both the pigments and the salts are detectable, but only the former are tested for ordinarily. In obstructive jaundice, relatively large amounts of bile pigments may be found in the urine. In hemolytic and in toxic jaundice, on the other hand, little or no bile pigment passes into the urine. The reason is that crystalloidal bilirubin can be readily excreted by the kidney if it is present in the circulating blood, as it is in obstructive jaundice. A colloidal complex is formed by the combination of bilirubin with a plasma globulin (see page 38) and is present in the blood in high concentration in hemolytic and toxic jaundice. The kidney cannot excrete it easily, since the kidney threshold for this large molecule is relatively high.

Urobilin, it will be remembered, is formed from bilirubin in the intestine by bacterial action. Urobilinogen is a product of the reduction of urobilin; this is also brought about by the intestinal flora. Urobilinogen may be absorbed from the gut and either be excreted by way of the bile after having been reconverted into bilirubin, or be excreted by the kidneys. The presence of urobilinogen in urine, therefore, is dependent upon the passage of bilirubin into the intestine.

Thus practically no urobilinogen will be found in the urine in obstructive jaundice, but in hemolytic and in toxic jaundice it occurs in appreciable quantities.

Urobilinogen may be detected by either Ehrlich's or Schlesinger's test. The former consists of adding to a few milliliters of urine a few crystals of para-dimethylaminobenzaldehyde and acidifying definitely with hydrochloric acid. In the presence of abnormal amounts of urobilinogen a cherry red color is seen. Normal urines give a negative test or a positive test only after heating. Watson has changed this into a quantitative procedure. Urobilin is first reduced to urobilinogen with ferrous hydroxide. The color is then developed with the Ehrlich reagent under definite conditions, and a colorimetric estimation is made, using a phenolsulfonphthalein standard for comparison. The Schlesinger test is performed as follows: to 10 ml. of urine is added a few drops of Lugol's solution to transform urobilinogen to urobilin. An equal volume of a saturated alcoholic solution of zinc acetate or zinc chloride is then added. The presence of urobilin is evidenced by a greenish fluorescence. This is best seen if the tube is placed in direct sunlight with a black background. If bile pigments are also present they should be removed first by adding about one-fifth of a volume of 10 per cent calcium chloride solution and filtering off the precipitated calcium-pigment compound.

**Urinary Calculi.**—The less soluble constituents of the urine sometimes precipitate out in the urinary tract. They may form minute particles or masses and be passed readily, or they may become larger aggregates, varying in size from "sand" or "gravel" to good-sized "stones." The substances of which they are composed are the same as those which may form sediments in the urine on standing; namely, uric acid and urates, calcium oxalate, calcium phosphate, calcium carbonate, and, very rarely, cystine, xanthine, and others.

These substances are ordinarily held in solution at body temperature in urine. Undoubtedly some of them are in a supersaturated state and it is probable that they are kept in solution because certain urinary colloids exert a "protective colloidal action." The colloids may be the urinary pigments, the traces of proteins, or other undetermined compounds. It is assumed that the crystalloids become insoluble either because of a change in the quantitative relationships (i.e., there is not enough protective colloid available) or because of a change in the degree of dispersion of the protective colloid. It is certain that the hydrogen ion concentration of the urine also plays a role.  $\text{Ca}_3(\text{PO}_4)_2$ , for example, is far less soluble in neutral urine than in acid, and is still less soluble at pH 8.0. Infection and diet may easily tend to change the pH of urine, and stasis may also be a factor in promoting the precipitation of salts. Certain other factors have been suggested as contributing to the formation of urinary calculi. Among them are hyperparathyroidism, hypervitaminosis D, and avitaminosis A. Kidney stones are quite prevalent in tropical countries, hence the suggestion that the overproduction of vitamin D by sunlight is a causative factor. The effect of high vitamin D producing a calcium imbalance coupled with a possible low vitamin A intake might induce the formation of calcium stones. An avitaminosis A has sometimes produced bladder stones in animals. Urinary calculi are often associated with hyperparathyroidism, which results in a removal of calcium salts from bone, with a rise in blood calcium and urinary calcium. However, many types of urinary calculi contain no calcium.

Uric acid and urate calculi are the most common of the *bladder* stones. They are always colored, being yellow to reddish-brown, and usually, but not always, have a smooth surface. The nuclei, or centers, of urinary concretions of other types are often composed of uric acid or urates.

Calcium oxalate stones are perhaps the next in frequency among bladder stones. They are dark brown to black, exceedingly hard, and usually rough, particularly the larger ones. This roughness is due to the protrusion of the sharp octahedral crystals. These are called the "mulberry" calculi. A small type is termed the "hempseed" calculus and may have a smooth surface. The majority of stones found in the kidney at operation are either oxalate or phosphate concretions.

Phosphate calculi are white to gray in color, usually rough, but sometimes smooth. They are more easily crushed than the first two types mentioned. They may be composed of mixtures of calcium phosphate, magnesium phosphate, and ammonium-magnesium phosphate in various proportions. Often urates and oxalates are also present. Calcium carbonate stones are less common in man than in herbivorous animals. They are rather small, white or gray, smooth spherical stones.

Cystine calculi may occur in cases of cystinuria. They are white, yellow, or greenish yellow, and rather soft. They are very rare. Even rarer are xanthine calculi. These, however, are harder than cystine stones and are brown or red in color.

### INBORN ERRORS OF METABOLISM

A condition of deranged metabolism which exists at birth and persists throughout life is known as an "inborn error of metabolism." In many instances the abnormality is a hereditary condition. The individual is unable either to utilize a certain type of nutrient or to produce from the ordinary foodstuff a particular physiologically important substance. Some of these have been discussed previously. They will be briefly summarized here.

Albinism is the inability to manufacture melanins, which are the dark pigments ordinarily deposited in the hair and skin. The condition is found in man as well as in other mammals.

Alcaptonuria is a disturbance in the intermediary metabolism of phenylalanine and tyrosine. Homogentisic acid is excreted in the urine. Upon exposure to the oxygen of the air this becomes very dark. Frequently "ochronosis" and arthritis occur as the patient matures. Ochronosis is a blackening of the cartilages of the individual. Alcaptonuria is a rather rare condition.

Cystinuria occurs in various degrees of severity. "Light" cases are not common but the very severe condition is quite rarely seen. Cystine itself, when fed to cystinurics, is metabolized apparently normally, but cysteine and methionine are excreted as cystine. Persons suffering from cystinuria are likely to have cystine calculi, which sometimes must be removed surgically from the kidneys.



Fructosuria is a very unusual state. Free or combined fructose of the diet is not converted into glycogen but is excreted. It seems to produce no harmful effects.

Pentosuria is another condition in which a sugar is excreted. Here the sugar is L-xyloketose. The amount excreted seems to bear no relation to the diet, and again we have an apparently harmless abnormality.

Hemophilia may also be included in the list of inborn errors of metabolism, since here one or more of the clotting factors either is insufficient in amount or does not possess the property required for normal clotting.

Porphyria is a disturbance in the metabolism of heme. A porphyrin is excreted in the urine, giving it a red color.

Tyrosinosis is an exceedingly rare anomaly in which the aromatic amino acids are eliminated as tyrosine or hydroxyphenylpyruvic acid.

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## Chapter 20

# THE CHEMISTRY OF RESPIRATION AND ACID-BASE BALANCE

Respiration is that physiological function which involves the exchange of gases between the body and the air. In the lungs oxygen passes from the air into the blood and carbon dioxide from the blood into the air. These are the two ends of the process, but in between these ends there occur many phenomena which are surprising in character. They include physical, chemical, and "nervous" changes. It is often difficult to set forth the stages in consecutive fashion, because the reactions are intricate, are closely interrelated, and are occurring simultaneously.

After the blood receives oxygen from the air it carries it to the tissues, where the oxygen is utilized in metabolic processes. The end products include inorganic and organic acids— $\text{H}_2\text{CO}_3$ ,  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$ , uric acid, lactic acid and others. The acids are largely neutralized by bases in the blood and tissue fluids, chiefly  $\text{NaHCO}_3$ , and thereby salts and  $\text{CO}_2$  are formed. Most of the salts and acids are excreted by the kidney and almost all of the  $\text{CO}_2$  by the lungs. The mechanisms of the processes will be discussed in this chapter.

### FLOW OF RESPIRATORY GASES

At this point it is pertinent to suggest that the gas laws be reviewed (Chapter 2). It follows from these laws that a given gas will tend to flow from a high partial pressure, or "tension," to a lower one, regardless of whether the gas is in gaseous form or is dissolved as a gas in a fluid. First, the composition of the gases present in inspired and expired air, and in the air present in the alveoli, will be considered. (Table XL.)

TABLE XL

AVERAGE COMPOSITION ON DRY RESPIRATORY AIR REDUCED TO STANDARD TEMPERATURE AND PRESSURE

	OXYGEN (PER CENT)	CARBON DIOXIDE (PER CENT)	$\text{N}_2$ , ARGON, ETC. (PER CENT)
Inspired air	20.94	0.04	79.02
Expired air	16.3	4.0	79.7
Alveolar air	14.2	5.5	80.3

The oxygen tensions (or partial pressures of oxygen) of these mixtures of gases are found by multiplying the total pressure by the percentage of oxygen in each case. Consequently the partial pressure of oxygen in inspired or atmospheric air is 20.9 per cent of the total pressure; that is, 159 mm. of Hg if the total pressure is assumed to be 760 mm. of Hg. Similarly the partial pressure of the oxygen in expired air would be 124 mm. Hg, and of alveolar air, 108 mm. Hg. It is evident that the direction of the flow of oxygen is toward the alveoli. Why do the alveoli have less oxygen than inspired air? Obviously because



oxygen must have been removed in the lungs and the reason for this is that the venous blood which is brought to the lungs has a low oxygen tension, namely, only 40 or 50 mm. Hg. This blood circulates through the capillaries of the lungs at astonishing speed. The combined thickness of the respiratory epithelium and capillary wall, which separates the blood from the air, is not over 0.004 mm. Every corpuscle is thus brought almost into actual contact with the alveolar air and conditions are excellent for rapid diffusion of gases. There is the added factor of the peculiar affinity of hemoglobin for gases. Consequently oxygen flows from the partial pressure of 108 mm. in the alveoli toward the 40 to 50 mm. in the venous blood, building it up in a flash to about 100 mm., the partial pressure of oxygen in *arterial blood*, the state in which it leaves the lungs.

Arterial blood, loaded with oxygen, at a partial pressure of about 100 mm., is carried to the muscles, spleen, heart, and other tissues. There, in the capillaries, it is separated from the tissue fluids by thin capillary walls and from the cells by their thin walls. There is reason to believe that the partial pressures of oxygen in tissue fluids are as low as 20 to 50 mm. and those of the cell contents about the same or less. Hence, the oxygen by physical forces alone would tend to flow out from the blood into the tissues. There are, however, other factors which lead in the same direction, factors which serve to dissociate oxyhemoglobin and to combine with the oxygen. As a result the blood comes out of the tissue capillaries and into the veins depleted of much of its oxygen, with a partial pressure of 40 to 50 mm. and goes back to the lungs for more  $O_2$ .

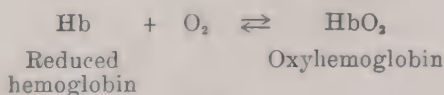
To summarize the flow of oxygen in relation to partial pressures: Oxygen in *atmospheric air* (159 mm.) flows toward *alveolar air* (108 mm.) and diffuses into *venous blood* (50 mm.). This is rapidly built up with the aid of hemoglobin to *arterial blood* (100 mm.), which gives oxygen to the *tissues* (50 mm.), becoming venous blood of about the same partial pressure (50 mm.), and thence back to the *alveoli of the lungs*.

Since  $CO_2$  forms  $H_2CO_3$  with water and reacts chemically with bases, the distribution of this gas is not entirely a physical matter. However, the direction of flow is also from higher to lower pressures as can be seen from the following figures. In venous blood the partial pressure is 46 mm. Hg and in the alveolar air it is 40 mm. Therefore, the tendency is to pass from the venous blood into the alveoli, to the expired air (20 mm.), to the atmospheric air (0.30 mm.). After the venous blood has lost  $CO_2$ , the partial pressure is down to 40 mm., at which level it goes to the tissues. Here the  $CO_2$  is high, with estimated partial pressures of 50 to 70 mm. Consequently  $CO_2$  flows into the arterial blood as it courses through the capillaries, bringing its  $CO_2$  up from 40 to about 46 at the same time that its oxygen is going down. The venous blood now passes to the lungs again to unload  $CO_2$ .

### THE CARRIAGE OF OXYGEN

If arterial whole blood is analyzed for its content of oxygen, it will be found to contain from 18 to 20 volumes per cent, when corrected to 0° C. and 60 mm. Hg. If the plasma is analyzed apart from whole blood, its oxygen content is about 0.3 volumes per cent; that is, 100 ml. of whole blood carries from

18 to 20 ml. of oxygen, whereas if it contained no corpuscles it could carry only 0.3 ml. The oxygen-carrying capacity of whole blood is therefore sixty or more times greater than plasma because of the presence of the erythrocytes with their hemoglobin. If blood contained no hemoglobin, Barcroft says, we would have to have over 150 kg. of plasma in our blood system. That is, the vascular system alone would amount to more than twice the weight of the body, and the organism would be unable to cope with the weight of its own blood. This indicates what a remarkable substance hemoglobin is. This power of combining with oxygen and of releasing it is not the only role played by this ferro-protein, but it is the most important. The percentage of saturation with oxygen varies with several factors and is shown in "dissociation curves," since this is a reversible reaction:



A series of such curves is illustrated in Fig. 72. These show the influence of different  $\text{CO}_2$  pressures in the dissociation of oxyhemoglobin of human blood.

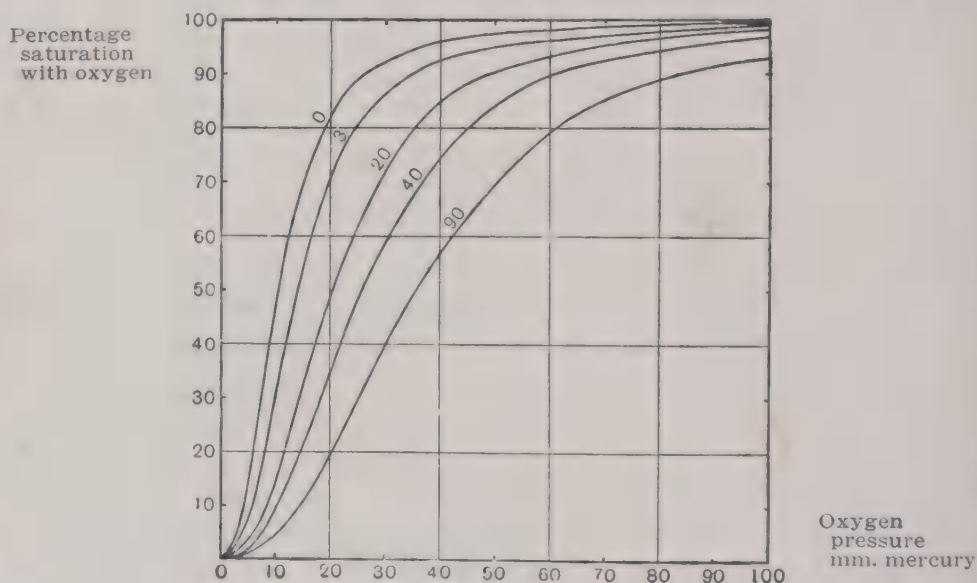


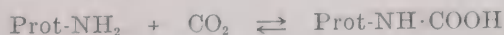
Fig. 72.—Dissociation curves of human blood exposed to 0, 3, 20, 40, and 90 mm.  $\text{CO}_2$ . Ordinate, percentage saturation with oxygen; abscissa, oxygen pressure. (From Barcroft, Joseph, in Bard, P.: *MacLeod's Physiology in Modern Medicine*, ed. 9, St. Louis, 1941, The C. V. Mosby Co.)

Thus, if curve 0 is followed from right to left, when no  $\text{CO}_2$  is present, the blood is fully saturated with oxygen at 100 mm.  $\text{O}_2$  pressure; at 40 mm.  $\text{O}_2$  pressure is about 96 per cent saturated; at 20 mm.  $\text{O}_2$  pressure it is 83 per cent saturated; and at 0 mm.  $\text{O}_2$  pressure it contains no oxygen. In other words, as the  $\text{O}_2$  tension increases, the above reaction proceeds to the right and more oxyhemoglobin is formed, as in the lungs. When the oxygen tension decreases, as in the tissues, the reaction goes toward the left and more oxygen is liberated. It is seen that as the  $\text{CO}_2$  tension, or partial pressure, is increased, the dissociation curves are shifted to the right. This means that if more  $\text{CO}_2$  is present, the hemoglobin can hold less  $\text{O}_2$ . Comparing the same curve (curve 0)

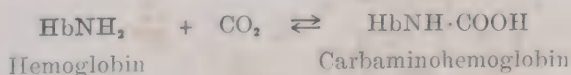
with no  $\text{CO}_2$  and the one at 40 mm.  $\text{CO}_2$  (curve 40), it is seen that at 100 mm.  $\text{O}_2$  pressure both are practically completely saturated with oxygen. That is, the hemoglobin is almost all present as oxyhemoglobin. At 90 mm.  $\text{O}_2$  pressure, which is the pressure in the arteries, they are still nearly the same, the curve 0 being about 99 per cent and curve 40 being about 95 per cent saturated. At 40 mm.  $\text{O}_2$  pressure (the  $\text{O}_2$  pressure of venous blood), the 0 curve still shows about 95 per cent saturation while curve 40 is down to 72 per cent saturation. That is, the presence of 40 mm.  $\text{CO}_2$  has caused the oxyhemoglobin to dissociate 23 per cent of its oxygen. Arterial blood has an approximate  $\text{CO}_2$  tension of 40 mm. and venous blood about 46 mm., as stated. The high venous  $\text{CO}_2$  pressures of the tissues (50 to 70 mm.) would cause the oxyhemoglobin to dissociate still more easily. Other acids have similar effects. It is thus evident that the effect of  $\text{CO}_2$  pressure is just the opposite to  $\text{O}_2$  pressure and both have a desirable physiological effect. In the tissues with low oxygen and high  $\text{CO}_2$  tensions oxyhemoglobin dissociates more readily and oxygen is available for tissue needs. In the alveoli, the oxygen tension of the air is high and there is no difficulty in forming oxyhemoglobin despite the high  $\text{CO}_2$  pressure (see tops of all curves).

### THE CARRIAGE OF CARBON DIOXIDE

As has been seen, carbon dioxide tends to flow from the tissues to the venous blood and from the venous blood in the lungs into the alveoli. But the carriage of  $\text{CO}_2$  and its elimination in the expired air is not entirely a question of pressure. In fact, this is one of the least important factors. Of the 50 to 60 volumes of  $\text{CO}_2$  per 100 ml. of blood, only 2 to 3 ml., or about 5 per cent, are in solution and exerting a tension. This is often written in the hydrated form,  $\text{H}_2\text{CO}_3$ , although over 99 per cent of dissolved  $\text{CO}_2$  is not hydrated. If all of it were in solution in an aqueous medium, the pH would be about 4.0, which is far on the acid side and would mean death to the tissues. Since the pH of the plasma varies only from pH 7.3 to pH 7.5 normally (and but little more abnormally), it is evident that the major part of the  $\text{CO}_2$  must be in combined form. Most of it—over 90 per cent—is in the form of bicarbonate, some in the red cells, and some in the plasma and tissue fluids. Only about one-half of one per cent is present as carbonate. Another fraction, about 3 or 4 per cent, is present as carbamino compounds, formed with proteins, the free amino groups of which react with  $\text{CO}_2$ .



By far the major portion of this fraction is in the red cells, because hemoglobin is the most abundant protein in blood. The resulting carbamino compound of hemoglobin is often called carbaminohemoglobin.



The direction of this reaction is determined almost entirely by the proportion of oxyhemoglobin present in blood and not by the level of the  $\text{CO}_2$  tension. Oxyhemoglobin is a more acid substance than reduced hemoglobin. When more



oxyhemoglobin is present, the reaction goes to the left; i.e., more  $\text{CO}_2$  is released; and on the venous side, when hemoglobin is in the less-oxygenated, less-acid state, more  $\text{CO}_2$  is combined. Hence the blood can carry more  $\text{CO}_2$  as carbamino hemoglobin on the venous side. At the instant of oxygenation in the lungs, the more acid oxyhemoglobin forces the carbaminohemoglobin to unload some of its  $\text{CO}_2$  into the alveoli. This, however, is only a small part of the  $\text{CO}_2$  story.

### The Chloride Shift

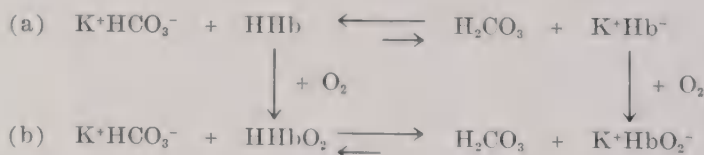
A few factors which play important roles must be mentioned first. Although we know that the reaction



takes place readily in both directions, the rapidity with which this occurs in the body in certain locations has led biochemists to wonder whether it was not catalyzed by some enzyme. A few years ago this was found to be so. Meldrum and Roughton discovered that an enzyme, which catalyzes the above reaction, is present in high concentration in red cells. Thus carbonic acid can be formed with extreme speed and it can be decomposed equally rapidly by the same enzyme under appropriate conditions. Another factor is the permeability of the red cell. It is impermeable to hemoglobin and to the plasma proteins, and is permeable to water,  $\text{CO}_2$ , bicarbonate, chloride, hydroxyl, sodium, potassium, and hydrogen ions (Sheppard). Most of the sodium ions are in the plasma and most of the potassium ions are in the cells. In the red cells a great deal of the hemoglobin is combined with potassium, the amount fluctuating in different parts of the cycle. With these facts in mind let us follow the courses of  $\text{O}_2$  and  $\text{CO}_2$ , into and out of the erythrocytes, and through the various parts of the respiratory cycle.

#### I. In the lungs:

(1) Oxygen enters the erythrocyte due to the higher pressure of oxygen in the lungs. Reduced hemoglobin becomes oxyhemoglobin as shown in (a) and (b).



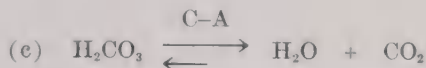
As oxyhemoglobin ( $\text{HHbO}_2$ ) is a stronger acid than reduced hemoglobin ( $\text{HHb}$ ), the equilibrium point is now shifted toward the right, converting bicarbonate ( $\text{HCO}_3^-$ ) to carbonic acid ( $\text{H}_2\text{CO}_3$ ). Thus an increased proportion of potassium ions becomes paired with oxyhemoglobin. The increase in acid strength of hemoglobin on oxygenation (or the reverse on deoxygenation), without change in blood pH, is called the isohydric change.

(2) The decrease in bicarbonate ( $\text{HCO}_3^-$ ) concentration in the erythrocyte leads to diffusion of bicarbonate from the plasma, where its concentration is higher, into the erythrocyte.

(3) To preserve electroneutrality, i.e., the equality in the number of positive and negative charges, some negative ion must leave the erythrocyte.

for each  $\text{HCO}_3^-$  ion entering it. Since the cell is permeable to  $\text{Cl}^-$ , which is present in sufficient amount, chloride ions diffuse out of the red cell. The total  $\text{B}^+$  (i.e.,  $\text{K}^+$  and  $\text{Na}^+$ ) content of the erythrocyte remains essentially constant.

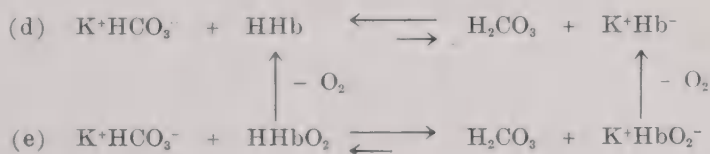
(4) The  $\text{H}_2\text{CO}_3$  formed in (b) quickly decomposes, in the presence of the carbonic anhydrase of the erythrocyte, to carbon dioxide and water thus:



(5) The low  $\text{CO}_2$  pressure in the lungs, compared with that of the blood arriving in the lungs, favors the escape of  $\text{CO}_2$  from the erythrocyte and plasma into the lungs, thereby shifting (c) and consequently (b) and (a) to the right. (As there are fewer osmotically active particles in the erythrocyte after  $\text{CO}_2$  escapes, some water leaves the erythrocyte.)

f. In the tissues:

(1) Because of the low oxygen pressure of the tissues, as compared with that of the lungs, the oxyhemoglobin of the erythrocyte gives up oxygen to the tissue fluids and becomes reduced hemoglobin, as shown in (d) and (e).

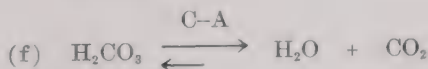


Reduced hemoglobin (HHb) is a weaker acid than oxyhemoglobin; consequently the equilibrium is shifted toward the left in (d), converting carbonic acid to bicarbonate. As a result, an increased proportion of potassium ions now becomes paired with bicarbonate.

(2) Now the increase in  $\text{HCO}_3^-$  concentration in the erythrocytes leads to diffusion of these ions from the erythrocytes into the plasma.

(3) Again a shift of the chloride ions in exchange for the bicarbonate ions occurs, but this time a  $\text{Cl}^-$  must enter the cell for each  $\text{HCO}_3^-$  which leaves it. The total  $\text{B}^+$  content of the red blood cell continues to remain essentially unchanged.

(4)  $\text{CO}_2$  diffuses from the tissues, where it is being formed in oxidative processes, into the plasma and then into the erythrocyte. Here in the presence of carbonic anhydrase some  $\text{H}_2\text{CO}_3$  is formed, shifting the equilibrium toward the left:

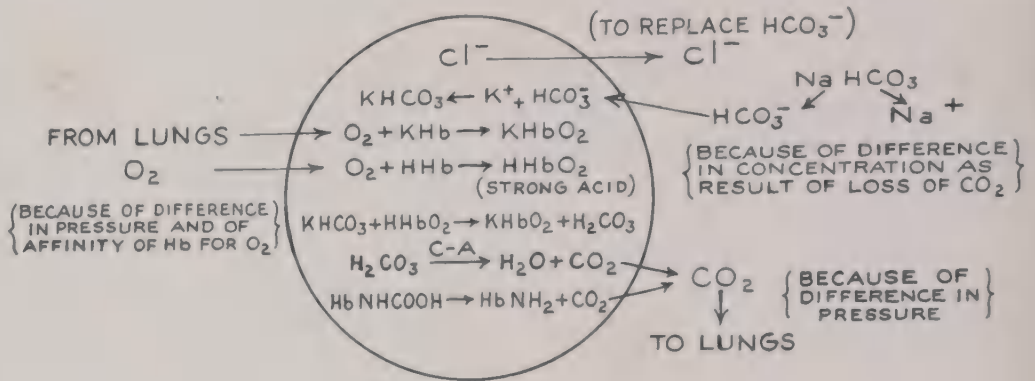


(Note: The equilibrium constant of (f) favors the existence of much more  $\text{O}_2$  than  $\text{H}_2\text{CO}_3$  at equilibrium. Carbonic anhydrase hastens the attainment of equilibrium and the formation of some  $\text{H}_2\text{CO}_3$ , as  $\text{CO}_2$  enters the erythrocyte.)

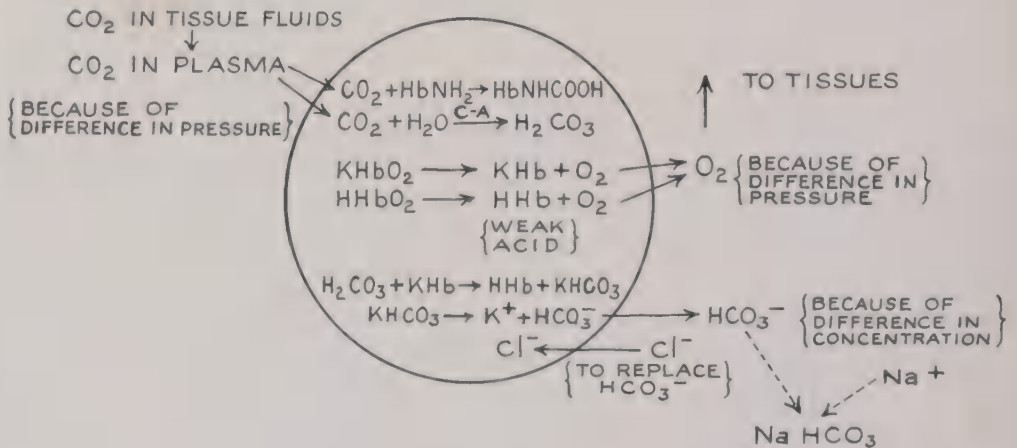
(5) The shifting of reaction (f) toward the left results in (d) and (e) also shifting toward the left. (As there are more osmotically active particles in the erythrocyte after  $\text{CO}_2$  enters it, some water now enters the red cell.)

Each phase of this cycle takes place with great rapidity. It is therefore necessary that a catalyst such as carbonic anhydrase be present and that conditions be optimum for rapid diffusion of gases. The extremely thin membranes, the small bore of the capillaries, allowing in parts of the system for only a single erythrocyte to pass through at a time, the temperature, all contribute to this end.

### IN CAPILLARIES OF LUNGS



### RED BLOOD CELL



### IN CAPILLARIES OF TISSUES

Fig. 73.—Diagrammatic representation of the chloride shift.

This phenomenon is known as the Hamburger phenomenon or "chloride shift" and is summarized diagrammatically in Fig. 73. Here C-A refers to carbonic anhydrase. H<sub>2</sub>CO<sub>3</sub> (or CO<sub>2</sub> gas) and Cl<sup>-</sup> always move in a direction opposite to HCO<sub>3</sub><sup>-</sup> in the Hamburger shift.



The increased acid strength of hemoglobin on oxygenation acts as though acid had been added to the red cell, liberating  $\text{CO}_2$ . Conversely, in the tissue capillaries, deoxygenation decreases the acid strength of hemoglobin, which, therefore, accepts  $\text{H}^+$  ions from the  $\text{H}_2\text{CO}_3$  entering the red cell. This allows most of the  $\text{CO}_2$  (or  $\text{H}_2\text{CO}_3$ ) from the tissues to be carried in the blood as bicarbonate,  $\text{B}^+\text{HCO}_3^-$ . The chloride-bicarbonate shift subsequently permits about 60 per cent of the  $\text{CO}_2$  from the tissues to be carried to the lungs as bicarbonate *in the plasma*. Venous blood has 4 to 10 per cent more "total  $\text{CO}_2$ " than arterial, i.e., 2 to 5 more "volumes per cent," or 1 to 2 mM. more per liter. About three-fourths of this extra  $\text{CO}_2$  in venous blood is carried as  $\text{B}^+\text{HCO}_3^-$ . Twice as much  $\text{B}^+$  for  $\text{B}^+\text{HCO}_3^-$  formation arises from the isohydric change of hemoglobin as is available from the ordinary buffering action of hemoglobin and plasma proteins on the invading  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ). The former does not change the pH of the plasma, while the latter lowers it slightly. The remaining one-fourth of the extra  $\text{CO}_2$  is carried as carbaminohemoglobin and as physically dissolved  $\text{CO}_2$  gas. The pH of venous plasma is 0.02 to 0.04 unit lower than that of arterial plasma, but venous plasma has about 1 or 2 mM. more of  $\text{B}^+\text{HCO}_3^-$  per liter than arterial plasma.

Hemoglobin is the most important buffer against any pH change that could result from  $\text{CO}_2$  entering the blood, mainly because the isohydric change of oxyhemoglobin to hemoglobin results in the conversion of most of the invading  $\text{CO}_2$  to  $\text{HCO}_3^-$ .

**Chemical Regulation of Respiratory Movements.**—The control of respiratory movements is considered in detail in textbooks of physiology and only a few words will be devoted to it here. There is some degree of voluntary control, but the regulation is chiefly involuntary and depends upon afferent impulses to the respiratory center, which thereupon sends its impulses to the various muscles involved. Chemical influences have much to do with some of these mechanisms. Excess of  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) in the blood stimulates the center directly, and, since fixed acids have the same effect, presumably it is due to a slight increase in hydrogen ion concentration (or decrease in the hydroxyl and carbonate ions) at the site of the center. Increases in  $\text{CO}_2$  of the inspired air raise the rate and depth of respiration and the result is a remarkable constancy in the percentage of  $\text{CO}_2$  in the alveolar air (about 5.5 per cent). Air or oxygen given for resuscitation should contain about 5 per cent  $\text{CO}_2$  to stimulate the respiratory center. Lack of oxygen has little effect unless it is very great. In that case respiration is increased, owing to responses of the carotid and aortic bodies, acting as emergency mechanisms.

Boyer and Bailey have shown that under basal conditions, that is, when the subject is at mental and physical rest, the concentration of the  $\text{CO}_2$  of the expired air (collected over a period of from five to twenty-five minutes) is constant for normal subjects. The range in a large number of determinations was found to be from 18.0 to 22.5 mm., and an average partial pressure of about 20.0 mm. is an acceptable clinical standard for both sexes and for all ages.

Low concentrations of  $\text{CO}_2$  in the expired air, indicating respiratory stimulation, would be expected in subjects with circulatory failure, acidosis, severe anemia, and certain forms of pulmonary disease. High concentrations would be expected in alkalosis and in depression of the respiratory center, as occurs after morphine or barbiturate administration. In cardiac

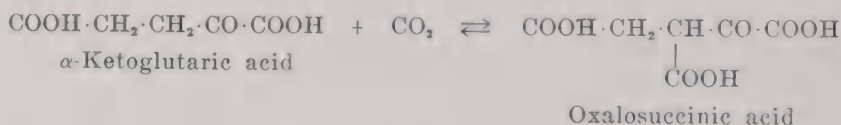
patients a definite relationship between degree of cardiac failure and  $\text{CO}_2$  concentration in the expired air was found. Those with slight limitation of physical activity had an average  $\text{CO}_2$  of 18.55 mm., those with marked limitation of physical activity had an average of 15.81 mm., while those who were unable to carry on any physical activity whatever without discomfort averaged 13.44 mm.

**Physiological Oxidations.**—It has been seen that hemoglobin combines with oxygen during the brief moment that the red cell spends in the capillaries of the lungs. About 95 per cent of the hemoglobin is united with oxygen when the arterial blood speeds to the tissues. In the tissue capillaries, because of the high  $\text{CO}_2$  tension and the low  $\text{O}_2$  tension, the dissociation of oxyhemoglobin is accelerated and oxygen flows into the plasma, diffuses through the walls of the capillaries into the tissue fluids and into the cells themselves. In and around the cells there occur those vital reactions whereby the digested and in some cases partly resynthesized food products, as well as fragments of protoplasm, are oxidized. In these processes energy is released and  $\text{CO}_2$  and  $\text{H}_2\text{O}$  formed as final products of some of these reactions (see Chapter 14).

**Carbon Dioxide Utilization in Animal Tissues.**—In the past it was commonly accepted that only plants utilize  $\text{CO}_2$  in photosynthetic and other processes; but that in animals  $\text{CO}_2$  is produced in the course of metabolism, is not utilized at all, and is excreted as an end product. In recent years this idea has had to be modified in view of the increasing evidence that  $\text{CO}_2$  can enter into synthetic reactions in the animal body. The incorporation of the carbon of sodium bicarbonate into glycogen has already been mentioned (page 417). A number of other reactions have been demonstrated, usually with the aid of isotopically tagged compounds. (Wood.) Evans and associates have shown that  $\text{CO}_2$  can be fixed by pigeon liver, by addition to pyruvic or fumaric acid. The product is oxaloacetic acid.



The enzyme which catalyzes this reaction is oxaloacetate- $\beta$ -carboxylase, and ATP is a coenzyme for the reaction. (Utter and Wood.) A similar reversible reaction by aid of a specific enzyme occurring in heart muscle, and also in liver, converts  $\alpha$ -ketoglutaric acid to oxalosuccinic acid. (Ochoa and Weisz-Tabori.)



In bacterial metabolism many other  $\text{CO}_2$  fixations have been found to occur and these presage the discovery of similar enzyme systems in mammalian tissues.

### ACID-BASE BALANCE

The carbon dioxide arising from the oxidation of metabolites is, as was just stated, fixed to some extent, but it is chiefly an excretory product. When converted into carbonic acid by union with water, it exerts a definite acid action. Other acids may come from the ash of foods. Therefore, much of the material in the chapter Mineral Metabolism and Water Balance is interrelated

with the present subject. Basic factors, too, come from foods and both acids and bases are thrown into the blood stream—usually more strong acid than strong base but sometimes the reverse. And yet the blood remains at the remarkably constant level of pH 7.3 to 7.5 at all times during health. For the accomplishment of this, the body has four “lines of defense”: (1) the buffer systems of the blood, tissue fluids, and cells, as well as minerals of bones, (2) the excretion or retention of  $\text{CO}_2$  by the lungs, (3) the excretion of an acid or alkaline urine, and (4) the formation and excretion of ammonia or organic acids. Thus the body's internal environment is maintained at a rather constant hydrogen ion concentration. This enables the various enzyme systems to operate under proper conditions, particularly in relation to each other.

### The Buffer Systems of the Blood

In Chapter 2 it was shown that the hydrogen ion concentration of a solution of a weak acid,  $\text{HIA}$ , and its salt,  $\text{B}^+\text{A}^-$ , is

$$[\text{H}^+] = K \frac{[\text{HIA}]}{[\text{B}^+\text{A}^-]}$$

where  $K$  is the dissociation constant, and  $[\text{H}^+]$ ,  $[\text{HIA}]$ , and  $[\text{B}^+\text{A}^-]$  or  $[\text{A}^-]$  the concentrations of hydrogen ions, of the acid, and of the salt, respectively. The hydrogen ion concentration of such a buffer pair will remain constant if the ratio of the numerator to the denominator remains constant. Slight additions of acid or base to buffers (or subtractions of either) have very little effect for reasons outlined previously, but large changes will, of course, make a decided difference. These relationships should be kept in mind.

In logarithmic form, the relationship is:

$$\text{pH} = \text{pK}' + \log \frac{[\text{B}^+\text{A}^-]}{[\text{HIA}]}$$

For  $\text{H}_2\text{CO}_3$ ,  $\text{pK}'$  is 6.1; for  $\text{B}^+\text{H}_2\text{PO}_4^-$ ,  $\text{pK}'$  is 6.8. Therefore, to keep pH at 7.4, the ratios of these acids to their salts must be kept constant. Substituting in the Henderson-Hasselbalch equation:

$$\text{For } \text{H}_2\text{CO}_3, \quad 7.4 = 6.1 + \log \frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$$

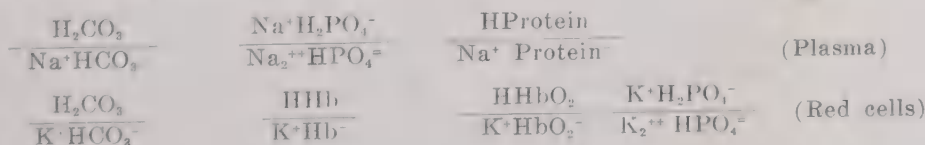
$$\text{or } 1.3 = \log \frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$$

$$\text{since antilog of } 1.3 = 20$$

$$\frac{\text{B}^+\text{HCO}_3^-}{\text{H}_2\text{CO}_3} = \frac{20}{1} \quad (\text{at pH } 7.4)$$

$$\text{Similarly, for } \text{B}^+\text{H}_2\text{PO}_4^-, \quad \frac{\text{B}_2^{++}\text{HPO}_4^-}{\text{B}^+\text{H}_2\text{PO}_4^-} = \frac{4}{1} \quad (\text{at pH } 7.4).$$

The buffers of the blood comprise:





The buffer pairs in the first line are chiefly or wholly in plasma and extracellular fluids and those of the second are chiefly or wholly in the red cells. (The sodium and potassium are not confined exclusively to the plasma or red cells, respectively. In the blood, of course, these buffers are all in equilibrium with each other. Therefore the estimation of any one buffer pair would be an index of acid-base equilibrium. Of all the pairs enumerated the  $\frac{\text{H}_2\text{CO}_3}{\text{B}^+\text{HCO}_3^-}$  is the most important, insofar as action against fixed, i.e., nonvolatile, acids is concerned. The phosphate pair, although more efficient as a buffer, is actually less effective, because of its low concentration in plasma. Plasma proteins play a greater buffering role than phosphates, but much less than hemoglobin. The bicarbonates neutralize more than 50 per cent of all acids stronger than  $\text{H}_2\text{CO}_3$ . Finally, in such neutralization  $\text{CO}_2$  is again formed and is readily eliminated as a gas by the lungs. (All salts, strong acids, and strong bases are virtually completely ionized. Thus  $\text{KHbO}_2$  is  $\text{K}^+\text{HbO}_2^-$ ,  $\text{HCl}$  is  $\text{H}^+\text{Cl}^-$ , and  $\text{NaOH}$  is  $\text{Na}^+\text{OH}^-$ .) An increase in  $[\text{H}^+]$  or  $[\text{H}_2\text{CO}_3]$  stimulates the respiratory centers to increase the rate and depth of respiratory ventilation. Similarly, an increase of  $[\text{OH}^-]$  or  $[\text{CO}_3^{=}]$  depresses respiratory ventilation. The lungs thus play a leading role in the minute-to-minute regulation of the pH of the blood and extracellular fluids.

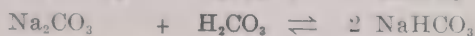
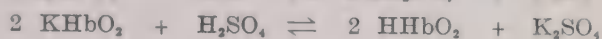
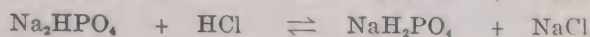
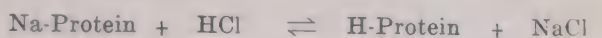
Examples of acids which can alter the acid-base balance include sulfuric, phosphoric, uric, lactic, acetoacetic, and  $\beta$ -hydroxybutyric acids. Their formation has been discussed in previous chapters.

As acid enters the blood, one of the buffer reactions which occur is:

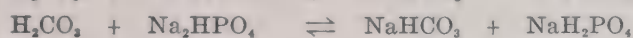


Here a strongly dissociated acid is transformed into the weakly dissociated acid  $\text{H}_2\text{CO}_3$ , thus changing the hydrogen ion concentration but little; i.e., by only as much as  $\text{H}_2\text{CO}_3$  is dissociated. This slight increase in the ratio  $\frac{\text{H}_2\text{CO}_3}{\text{BHCO}_3}$ , due to an increase in the numerator, can be rapidly brought down to normal because of the easy disposal of  $\text{H}_2\text{CO}_3$ , via the lungs.  $\text{H}_2\text{CO}_3$  is over 99 per cent  $\text{CO}_2$  gas. Thus any nonvolatile acid stronger than  $\text{H}_2\text{CO}_3$  can be buffered by  $\text{BHCO}_3$  as long as any bicarbonate is present. Consequently the plasma bicarbonate is a measure of the base remaining after all acids stronger than  $\text{H}_2\text{CO}_3$  have been neutralized. It represents the reserve of alkali available for the neutralization of such strong acids. Hence it has been called the "*alkali reserve*" by Van Slyke and Cullen. However, hemoglobinate plays an important role in buffering fixed acids, although not as great a one as bicarbonate. However, it is not directly measured when the "*alkali reserve*" ( $\text{CO}_2$ -combining power) is determined by the procedure described on page 549, but a decrease in plasma alkali reserve generally parallels a depletion of the reserve of buffering power represented by hemoglobinate.

Again it must be pointed out that the other buffers are in equilibrium with the bicarbonate pair and will react with acids (or bases), but to a lesser extent, because of their lower concentrations. For example:



In every case the strong acid or base is transformed into a weak one, and consequently the pH of the blood fluctuates very little. However, the acid formed in the largest amounts in the body is carbonic acid or its anhydride  $\text{CO}_2$ , and this cannot be buffered by bicarbonates. It can be buffered by serum proteins and by phosphates:



Both of these factors are of minor consequence. The most important buffer for carbonic acid is hemoglobin. Table XLI gives estimates of supplies of buffers in the various compartments of the body.

TABLE XLI  
BUFFERS OF BODY FLUIDS\*

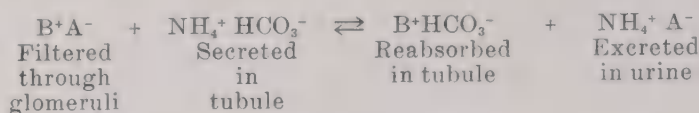
ESTIMATED PER CENT BUFFERING OF INVADING FIXED ACID OR ALKALI	CHIEF BUFFERS PRESENT	LOCATION OF BUFFER DEPOT
40	Partly $\text{BHCO}_3$ Partly unknown	Tissue cells
30	$\text{BHCO}_3$	Extracellular fluid except blood
13	$\text{BHb}$ and $\text{BHbO}_2$	Blood
17	$\text{BHCO}_3$	

\*From Van Slyke, D. D., and Cullen, G. E.: The Bicarbonate Concentration of the Blood in Asma; Its Significance, and Its Determination as a Measure of Acidosis, *J. Biol. Chem.* **30**: 9, 1917.

### The Role of the Kidney

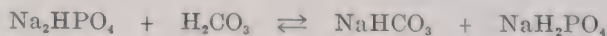
The kidney contributes to the maintenance of the "alkali reserve" and to constant blood pH by reabsorbing, secreting, and excreting acidic or basic substances, as the case may be. Moreover, while the lung can help excrete  $\text{CO}_2$ , it cannot restore the alkali reserve ( $\text{BHCO}_3$ )—something the kidney can do. Although phosphates are present in only small concentrations in the blood, they are concentrated by the kidney and are the principal buffers in urine as excreted. In acid urines, there is a relative excess of  $\text{BH}_2\text{PO}_4$ , and in alkaline urines,  $\text{B}_2\text{HPO}_4$ . There is also a considerable amount of  $\text{BHCO}_3$  in alkaline urines—notably  $\text{KHCO}_3$  from the metabolism of fruits and vegetables. Organic acids,  $\text{H}_2\text{CO}_3$ , and salts of organic bases contribute to some extent to the urinary acidity. In quite a different way the kidney has another effect on acid-base balance. It is the site of the formation of ammonia which is

secreted, probably as  $\text{NH}_4^+\text{HCO}_3^-$ , by the kidney tubules. This results in the conservation and restoration of  $\text{B}^+\text{HCO}_3^-$ , or alkali reserve, in the following manner. If a strong acid,  $\text{H}^+\text{A}^-$ , has been thrown into the blood, resulting in the replacement of some of the  $\text{B}^+\text{HCO}_3^-$  by  $\text{B}^+\text{A}^-$ , then the following occurs in the kidney to restore  $\text{B}^+\text{HCO}_3^-$  of the blood.



In acidosis the urinary ammonia rises considerably as a result of increased formation. It is evident that all the ammonia produced and excreted in this way takes the place of an equivalent amount of sodium, potassium, calcium or magnesium. Thus it prevents the loss of sodium and other cations in the urine to a very appreciable extent and saves them in order that they may remain at a rather definite concentration in the tissue fluids, a condition quite essential to preserving the constancy of the internal environment.

The mechanism of ammonia formation by the kidney has been discussed on page 374. The method whereby an acid urine is formed from a slightly alkaline blood plasma deserves some consideration. Urinary acidification is necessary to provide for the excretion of fixed acids and acid salts, and to restore alkali reserve. There are several current theories to account for the phenomenon of a glomerular filtrate of pH 7.4 being converted into a urine having a pH as low as 4.8. Some of these concepts are illustrated in Fig. 74 and may be summarized as follows: According to the *phosphate reabsorption theory* the glomerular filtrate contains as its significant constituents  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$ . The dibasic phosphate is reabsorbed by the renal tubule and returned to the blood, while the monobasic (acidic) salt remains in the tubule and becomes the titratable acid of the urine. The *carbonic acid filtration theory* maintains that the glomerular filtrate contains carbonic acid and sodium bicarbonate in addition to the two types of phosphate. There is also the assumption that the tubules can remain impermeable to  $\text{H}_2\text{CO}_3$  ( $\text{CO}_2$ ) as they actively reabsorb  $\text{NaHCO}_3$ . Thus the equilibrium,



is moved to the right as  $\text{NaHCO}_3$  is removed, leaving  $\text{NaH}_2\text{PO}_4$  in the urine. If the tubules are considered permeable to  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ), the  $\text{H}_2\text{CO}_3$  available from surrounding areas serves as an almost endless source of  $\text{NaHCO}_3$ , through the above equilibrium reaction, which is moved to the right as  $\text{NaHCO}_3$  is reabsorbed. This mechanism, the *carbonic acid filtration-diffusion theory*, can account for a greater urinary acidity than the preceding theory, which is limited by the amount of  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) filtered (Menaker; Peters and Van Slyke; Sendroy). The *tubular ionic exchange theory* postulates that the  $\text{H}^+$  ions are actively transported by the tubular cells into the urine in exchange for  $\text{Na}^+$  (and  $\text{K}^+$ ) ions of the glomerular filtrate, thus converting  $\text{Na}_2\text{HPO}_4$  into  $\text{NaH}_2\text{PO}_4$ , and  $\text{NaHCO}_3$  into  $\text{H}_2\text{CO}_3$ , the latter escaping from the tubular lumen

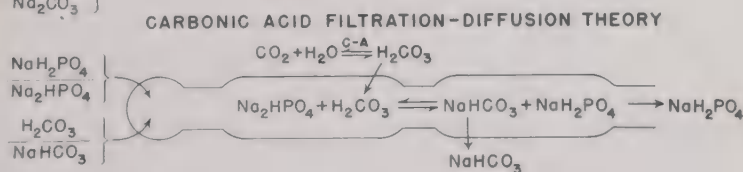
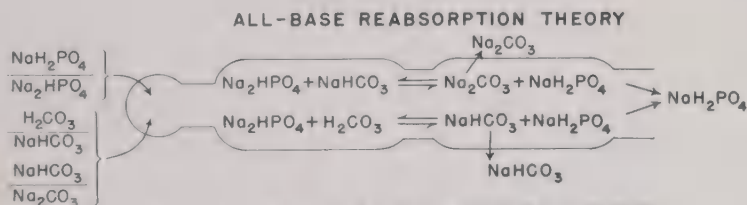
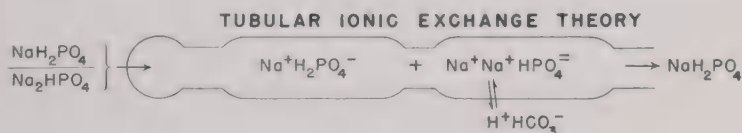
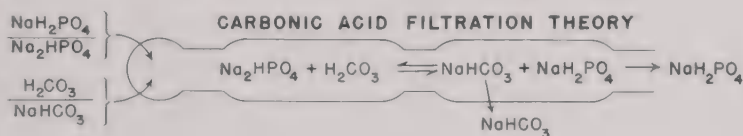
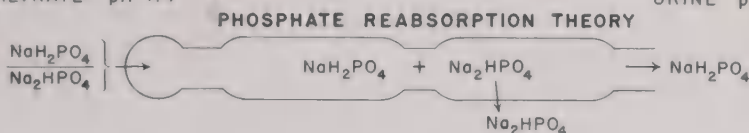


to the cells as  $\text{CO}_2$  (Pitts; Smith). In all these theories, carbonic anhydrase, in the tubular cells, assures the rapid conversion of  $\text{CO}_2$  ( $+\text{H}_2\text{O}$ ) to  $\text{H}_2\text{CO}_3$ .

According to the *all-base reabsorption theory*, the absorption of alkaline compounds, notably  $\text{Na}_2\text{CO}_3$  (carbonate) followed by  $\text{NaHCO}_3$  (bicarbonate), can completely explain any urinary acidity reported. (Menaker). As carbonate is reabsorbed, the equilibrium:

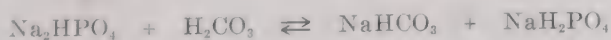


FILTRATE pH 7.4 URINE pH 4.8+



74.—Theories to account for acidification of urine. (Upper three parts from Pitts, R. F., and Alexander, R. S.; *Am. J. Physiol.* **144**: 239, 1945.)

moved to the right, converting  $\text{Na}_2\text{HPO}_4$  to  $\text{NaH}_2\text{PO}_4$ . Subsequent reabsorption of  $\text{NaHCO}_3$  completes the acidification process by converting more  $\text{Na}_2\text{HPO}_4$  into  $\text{NaH}_2\text{PO}_4$  as  $\text{NaHCO}_3$  reabsorption moves the following equilibrium to the right:



Three times as much bicarbonate as carbonate is reabsorbed in this postulated mechanism. ( $\text{H}_2\text{CO}_3$ , present in the carbonic anhydrase-rich kidney, readily reacts with reabsorbed  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{Na}_3\text{PO}_4$ , or  $\text{NaOH}$ .)

In all the acidifying mechanisms cited,  $\text{B}^+$  instead of  $\text{Na}^+$  might be used.

The kidney also protects the organism against an excess of alkali. An alkaline ash diet results in the production of  $\text{KHCO}_3$  and  $\text{K}_2\text{HPO}_4$ . The kidney produces an alkaline urine by excreting, i.e., not reabsorbing,  $\text{BHCO}_3$  and  $\text{B}_2\text{HPO}_4$ ; it might here reabsorb  $\text{BH}_2\text{PO}_4$ . When excreting an alkaline urine, the kidney also excretes much more organic acid, notably citric acid, essentially in the salt form, at this pH range.

As the ingestion or formation of fixed acids results (by interaction with  $\text{BHCO}_3$ ) in the formation of volatile  $\text{CO}_2$ , which is readily eliminated by the lungs, whereas no base can be exhaled in a similar manner, and as the kidney can form and excrete much ammonia, the organism is better equipped to combat the invasion of acid than alkali.

### ACIDOSIS AND ALKALOSIS

Peters and Van Slyke define acidosis as "an abnormal condition caused by the accumulation in the body of excess of acid or by the loss from the body of alkali." Similarly alkalosis is "an abnormal condition caused by the accumulation in the body of excess alkali or by the loss of acid." Ordinary amounts of acid or of alkali are taken care of by the mechanisms just considered. That is, ordinarily the ratio  $\frac{\text{H}_2\text{CO}_3}{\text{B}^+\text{HCO}_3^-}$  of the equation  $[\text{H}^+] = K \frac{[\text{H}_2\text{CO}_3]}{[\text{B}^+\text{HCO}_3^-]}$  is kept constant at about  $\frac{1}{20}$  and since this is in equilibrium with all the other sets of buffers, the pH remains at 7.3 to 7.4. Now in acidosis or alkalosis it may also be kept constant. If the acidosis is due to an increase of the numerator, a concomitant and sufficient increase in the denominator will hold the ratio constant. If it is due to a loss of alkali, i.e., a diminution of the denominator, a simultaneous decrease in the numerator will have the same effect. Similarly an alkalosis arising from an increased amount of alkali may be compensated for by an increased production of  $\text{H}_2\text{CO}_3$ , and a decrease in the numerator may be followed by a decrease in the denominator. In all of these four conditions the pH will not change. Such courses of events result in "compensated acidosis or alkalosis." When the ratio actually changes and the pH is outside of the normal range, the term "uncompensated" is used. However, long before any abnormally great deviation in the pH occurs, the bicarbonate content changes. This is easily detected by determining the carbon dioxide combining power of blood plasma.

The normal concentration of  $\text{BHCO}_3$  in plasma is about 0.025 molar or 25 mM. per liter. Plasma  $\text{H}_2\text{CO}_3$  (mainly  $\text{CO}_2$  gas) is about 1.2 millimolar. Plasma  $\text{B}_2\text{CO}_3$  is 0.1 millimolar. The total  $\text{CO}_2$  (mainly bicarbonate) is thus about 26 millimolar. When measured in the laboratory by the addition of acid to plasma, the amount of  $\text{CO}_2$  liberated from the plasma represents mainly  $\text{BHCO}_3$ , or the "alkali reserve," and is reported in "volumes per cent"—the number of ml. of  $\text{CO}_2$  that would be liberated from 100 ml. of plasma. From the gas laws, we know that 22.4 ml. of a perfect gas represents one millimol of the gas. If 1 ml. of plasma gave 0.224 ml. of  $\text{CO}_2$  on acidification, it would be reported as 22.4 volumes per cent and would mean that there are 10 mM. of total  $\text{CO}_2$  in 1 liter of plasma. To convert "volumes per cent" to mM. per liter, divide by 2.24.

**Carbon Dioxide Combining Power of Blood Plasma.**—The principles involved in this determination are as follows: The blood is taken from a vein by syringe and is transferred to an oxalated centrifuge tube. After centrifuging, the plasma is placed in a separatory funnel and exposed to an atmosphere whose carbon dioxide tension is approximately that of sea-level air. A measured volume of this saturated plasma is then placed in a Van Slyke carbon dioxide apparatus, acidified, and subjected to negative pressure. This treatment liberates the  $\text{CO}_2$  from bicarbonate as well as the  $\text{CO}_2$  in solution. The bubble is returned to the calibrated part of the apparatus and measured at atmospheric pressure. Normal blood plasma combines with from 50 to 70 ml. of  $\text{CO}_2$  per 100 ml. If the buffering power is depleted because acids have been thrown into the blood in excessive amounts (acidosis), less  $\text{CO}_2$  can be taken care of and the  $\text{CO}_2$  combining power will be lower. In alkalosis more  $\text{CO}_2$  can be combined of course. If a patient has received  $\text{NaHCO}_3$ , a false picture may result because this will increase the volume of  $\text{CO}_2$  itself, although the fundamental metabolic condition of the patient may be unchanged and this possibility must be kept in mind. In general, a  $\text{CO}_2$  combining power of over 70 ml. per 100 ml. indicates alkalosis; 50 to 70 ml. is normal; 41 to 50 ml. indicates mild acidosis; 31 to 40 ml. indicates moderate acidosis; and 30 ml. or less indicates severe acidosis. For details of this procedure, the reader is referred to laboratory manuals.

### Disturbances in Acid-Base Balance

If it is kept in mind that acid-base balance depends upon the ratio  $\frac{\text{H}_2\text{CO}_3}{\text{HCO}_3^-}$ , it is seen that there are nine possible states which may occur in the blood (Van Slyke). They are, in the first place, a normal relationship. Then there are excesses of either numerator or denominator and deficits of either. That is, there are four deflections from the normal, and since each may be compensated or uncompensated, they total eight.

**I. Primary Alkali Deficit.**—In this condition the bicarbonate is diminished as a result of increased production, ingestion, or retention of acid. The increased production occurs in diabetes mellitus and in certain other metabolic disturbances. Here such acids as beta-hydroxybutyric are not utilized in the normal manner and they therefore make inroads on the alkali reserve. The ingestion of mineral acids, as might occur from the administration of  $\text{HCl}$  in gastric disturbances, has the same effect. Infantile diarrhea may result in loss of base. In nephritis the kidney may not excrete acids in sufficient amounts and retention therefore occurs. Except in the case of retention, primary alkali deficit leads to increased elimination of acid in the urine. There is also a rise in urinary ammonia. Respiration is increased in order to get rid of  $\text{CO}_2$  faster. All of these compensatory mechanisms tend to reduce the  $\text{H}_2\text{CO}_3$  (numerator). If the reduction is sufficient to parallel that of the bicarbonate (denominator), the ratio remains constant and a *compensated acidosis* results. If not, it is *uncompensated*; the pH falls and the patient may go into coma. This has also been called "metabolic acidosis."

**II. Primary Alkali Excess.**—The ingestion of excessive amounts of "bicarbonate of soda" is about the only example of increasing the bicarbonate fraction in an absolute manner. However, removing acid from the body has the same result, relatively. An example of the latter is excessive vomiting as it occurs in pyloric obstruction, with consequent loss of gastric  $\text{HCl}$ . The physiological mechanisms for combating this are an increased excretion of alkali by the



kidney and, at the same time, a diminished formation of ammonia. Respiration is depressed so that loss of  $\text{CO}_2$  is very low. If these physiological efforts are successful in holding the ratio constant, again there is a compensated condition of alkalosis with few, if any, untoward symptoms. However, if it is uncompensated and the pH rises to an abnormal level, the alkalosis is grave and tetany may occur. Tetany is a condition which may arise from other causes besides severe alkalosis. Neuromuscular excitability is the chief symptom in man, and even convulsive seizures occur in children as they do in lower animals. "Metabolic alkalosis" is another term applied to this type.

**III. Primary  $\text{CO}_2$  Excess.**—This is caused by any obstruction to respiration or depression of it. The former may occur in pneumonia or emphysema and the latter from depression of the respiratory center as a result of toxic doses of morphine or other respiratory depressants. Under these conditions, usually the lack of oxygen (anoxia) is more to be feared than the acidosis. However, it is an acidemia, and the compensatory mechanisms are an increase in the bicarbonate and a rise in urinary acid and ammonia. This leads to high  $\text{BHCO}_3$  with acidemia. Again this may be either compensated or uncompensated. This has sometimes been designated "respiratory acidosis" despite the high  $\text{BHCO}_3$ .

**IV. Primary  $\text{CO}_2$  Deficit.**—A loss of  $\text{CO}_2$  may occur when respiration is stimulated in some abnormal manner. Examples of this are more common than are usually believed. Fever and hot baths were the two most usual stimulants cited, but two others have more recently been brought to the attention of observers. One is the lack of oxygen existing at high altitudes. When this is very great, it increases the rate of respiration and  $\text{CO}_2$  is eliminated more rapidly. A second factor is anxiety or hysteria. The mental state results in hyperventilation, also, and the two factors may operate together in airplane passengers. In the army the "hyperventilation anxiety syndrome" was said to be a rather common condition in hospitalized cases in wartime (Lewis). It is often difficult to recognize. Primary  $\text{CO}_2$  deficit is, of course, an alkalemia which usually becomes compensated by a reduction of urinary ammonia formation and increased excretion of bicarbonate. A common term for this is "respiratory alkalosis," despite the low  $\text{BHCO}_3$ .

From a consideration of these conditions it must be evident that the determination of the alkali reserve alone will not always give a true picture of the condition. Sometimes a pH determination is also needed. For instance in an uncompensated  $\text{CO}_2$  deficit there is an alkalemia, due to a loss of volatile acid. In the attempt to compensate for the reduction in carbonic acid, there is, as stated, an increased excretion of bicarbonate. Thus we have a lowered blood bicarbonate with an alkalemia. On the other hand, in an uncompensated  $\text{CO}_2$  excess, the attempt of the body to compensate is the production and hoarding of bicarbonate. The blood will actually be more acid (acidemia) despite the presence of increased bicarbonate. These, of course, are extreme cases, but their implications are important. In III and IV above, acidemia or alkalemia refers to low or high pH, respectively.

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## Chapter 21

# ENERGY METABOLISM

The demonstration of the laws of conservation of matter and energy has been made again and again for animate as well as inanimate matter. Matter is transformed chemically and physically but none is lost and none gained; energy is changed from one form to another, but, here too, there is neither loss nor gain. When the products derived from foods are oxidized, there is an evolution of heat, in much the same way as when a substance is burned outside of the body. The "combustion" is not as intense, and, as has been seen, is accomplished with the aid of complicated enzyme reactions. But in the end they are oxidations and yield heat or other forms of energy. It was the great French scientist Lavoisier (1743-1794) who first demonstrated that animal heat is derived from oxidations of essentially the same sort as any other oxidation. He also showed that the amount of heat produced by the oxidation of a certain amount of carbon in an animal was equivalent to that produced by the combustion of the same amount of nonliving carbon.

In earlier chapters the material phases of metabolism, the building up and breaking down of tissues, were studied. Now the energy transformations involved in those processes will be discussed. Both phases make up *total metabolism*.

### HEAT REGULATION OF THE BODY

The transformation of the potential energy of the food into muscular contractions and other forms of kinetic energy by the animal body is, from a mechanical standpoint, more efficient than in most man-made machines. About 18 to 22 per cent of our food may be converted into mechanical energy, the energy of work, etc., as compared with from 9 to 19 per cent for the steam engine and 20 per cent for the gasoline motor. The Diesel engine, however, is more efficient since it transforms from 29 to 35 per cent into kinetic energy. The rest of the energy transformed from food, about 80 per cent, is liberated as heat. Usually the body is warmer than the surrounding atmosphere and loses heat to it. Sometimes, however, the reverse is the case, the atmospheric temperature being higher than that of the body, and the organism has the burden of losing heat, which is constantly being produced, against this gradient. When the environment is above body temperature, all of the excess heat must be eliminated by the evaporation of water. Nevertheless, the body temperature of the human being is remarkably constant. There are, to be sure, slight normal variations. In the early morning, between 2 and 5 A.M., the temperature is lowest, rising gradually to its highest point between 5 and 8 P.M. During sleep the temperature always goes down slightly. These variations do not exceed 1.8° F.; i.e., throughout the day, under ordinary conditions of work and rest, in



normal adult, the rectal temperature may range from 97.3 to 99.1° F. The temperatures of different parts of the body vary—that of the rectum is higher than that of the mouth, and that of the mouth higher than the temperature of the skin. In the normal adult female there is a rather regular temperature rhythm dependent upon the menstrual cycle. During the period between menstruation and ovulation there is a slow fall in body temperature, with occasionally a steeper drop just before ovulation. Immediately thereafter there is a sharp rise to a flat or slowly ascending plateau. Shortly before the onset of menstruation, the temperature rapidly falls again. At the menopause this ovarian temperature cycle ceases. Muscular work causes an increased heat production, of course, but this usually changes the temperature of the body but little because of the effectiveness of the heat-regulating mechanism. The temperature of the atmosphere usually does not affect body temperature, but prolonged hot or cold baths can raise or lower body temperature, respectively.

Heat is produced both in oxidative and in nonoxidative reactions in all tissues of the body, but chiefly in the muscles. There is, of course, some heat produced in digestion and in the various chemical reactions occurring in the liver and other organs. The contractions of nonstriated muscle, as in peristalsis, must give rise to some heat, and the heartbeat also accounts for a considerable amount. By far the largest factor, however, is that produced by the skeletal muscles. Even during rest, between periods of activity, they have a large energy requirement, and during work this may be increased enormously. When the external temperature is lowered, the muscles are called upon to produce more heat by shivering. Glands of internal secretion, particularly the thyroid, play a role in heat production by altering the rate of metabolism. In all of these ways heat production is affected, and they are thus a part of the heat-regulating mechanism.

On the other hand, there are several factors which regulate heat control by modifying the loss of heat from the body. There are three main paths by which heat is lost: the skin, the lungs, and the excretions. At least 85 per cent of the heat loss is from the skin. This occurs by conduction, radiation, convection, and evaporation. The relative amounts lost by these different processes will vary with the condition of the body and its environment. The amount of heat loss is affected by the clothing, the temperature of the air and walls of the room, the humidity, and the movement of the air are all important factors. Heat loss by way of the lungs is partly by vaporization and partly by convection. The amount of heat transferred or lost from the body in feces and urine is relatively small.

The regulation of heat loss is partly voluntary and partly involuntary. The former is seen in the various devices adopted for warming the body by clothing, heating our buildings, warming our food and drink. The involuntary regulatory machinery includes the vasomotor mechanism and the secretion of sweat. When the external temperature is high, the cutaneous blood vessels dilate and the abdominal vessels constrict and more blood is thereby exposed to the cooling influence of the external environment, which, although warm, is

almost invariably cooler than body temperature. The cooled blood is brought to the interior of the body, where it is again warmed and brought again and again to the surface for cooling. The sweat glands are stimulated to increase their secretion when heat loss from the skin by physical means is insufficient. Then the evaporation of sweat becomes a cooling factor. If the secretion is extremely rapid, or if the external temperature and humidity are high, evaporation will not keep pace with secretion and beads of perspiration will form.

The control of heat production and heat loss is vested in the central nervous system. The central mechanism for regulating heat production is located in the posterior hypothalamus, while that for heat loss is in the anterior hypothalamus.

### Measurement of Heat

In physiological studies heat is measured in *large calories*, "Cal," or "C." A Calorie is the amount of heat needed to raise one kilogram of water  $1^{\circ}$  C. usually from  $15$  to  $16^{\circ}$  C. A bomb calorimeter is used to determine the caloric value of a given substance, a food for example. This is a metal vessel in which the weighed food is ignited in an atmosphere of oxygen by means of an electric spark. It is surrounded by a measured volume of water. The increase in temperature of the water multiplied by its weight gives the number of calories liberated by the combustion of the food. By such measurements it has been found that, on an average:

1 Gm. carbohydrate yields	4.1 Cal.
1 Gm. fat yields	9.4 Cal.
1 Gm. protein yields	5.6 Cal.

These figures are to be considered typical of the caloric equivalents of foods ordinarily present in the diet of man. But it should be remembered that specific members of each class may have values somewhat at variance with these typical or average values. For example, the 4.1 figure is applicable to starch,  $(C_6H_{10}O_5)_n$ , but glucose,  $C_6H_{12}O_6$ , produces only 3.8 Calories per gram. Individual fats also vary in their heat equivalents; olive oil yields 9.384 Calories, and butterfat 9.179 Calories per gram. When the same substances are utilized by the body, it can be shown that carbohydrates and fats have the same caloric values, but proteins give rise to only 4.1 Cal. instead of 5.6. The reason for this is that the proteins are not completely oxidized in the body since the urea, to which they give rise, could be oxidized still further in the bomb calorimeter. For practical purposes, the following approximate figures are usually employed when dealing with animal calorimetry:

1 Gm. carbohydrate yields	4 Cal.
1 Gm. fat yields	9 Cal.
1 Gm. protein yields	4 Cal.

The heat produced by the animal body can similarly be directly measured by placing the animal in a calorimeter. It can also be estimated indirectly by measuring the amount of oxygen retained by the body and the carbon dioxide excreted, and calculating the amount of heat which these values represent. The

direct method is far more accurate but entails the construction of complicated and costly apparatus. Therefore it is used in relatively few institutions.

**The Atwater-Rosa-Benedict Calorimeter.**—In Fig. 75 is shown a diagram of the Atwater-Rosa-Benedict calorimeter. In such an apparatus not only is the heat measured, but the oxygen absorption and carbon dioxide output can also be determined. Thus the direct and indirect methods may be employed simultaneously and may be compared with each other. The animal, or man, occupies a chamber which has a double copper wall enclosed in an insulated wall. The heat lost from the body is removed by a coil of pipe through which water flows. This is shown in the figure as a single tube with a thermometer, "T," at the inlet and another at the outlet. The number of calories lost from the body is calculated from the difference in temperature and the volume of water flowing

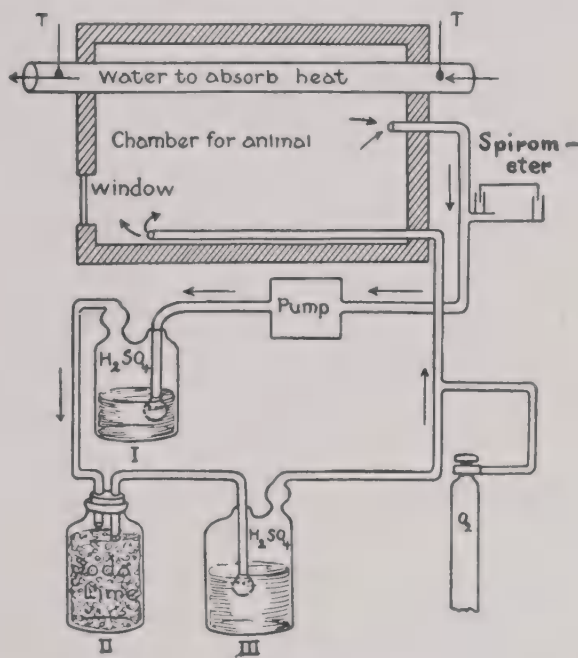


Fig. 75.—Diagram of Atwater-Rosa-Benedict respiration calorimeter. Heat produced by the body is absorbed by a coil of pipe through which water flows (here shown as a single tube).  $T$  and  $T$  are thermometers registering the temperature of the inflowing and outflowing water, the volume of which can be measured. This permits the calculation of "sensible heat." The pump blows the air in the direction of the arrows. The spirometer shows contraction of total air volume as the animal uses  $O_2$ . A corresponding amount of  $O_2$  is delivered from the weighed  $O_2$  cylinder. The increase in weight of Bottle I gives the water lost by evaporation and from this the latent heat of evaporation may be computed. The increase in weight of bottles II and III give the  $CO_2$  evolved. (From Bard, P.: *Macleod's Physiology in Modern Medicine*, ed. 9, St. Louis, 1941, The C. V. Mosby Co.)

through the coil. In addition to this, the heat removed as latent heat of evaporation must be computed. This is done by absorbing the moisture from the air leaving the calorimeter and weighing it. Each gram of water vaporized requires 80 Cal. at  $30^\circ C.$ , the temperature of the skin. Other losses of heat from the calorimeter are prevented by an elaborate system for keeping the inner and outer copper walls at the same temperature so that heat cannot flow in either direction. The air is circulated by a pump in the direction indicated by the arrows. As the subject uses up the oxygen from the air, the spirometer indicates the decrease in volume. Oxygen is then added from the weighed cylinder and the difference in



weight gives the amount of oxygen used. The bottles of sulfuric acid and soda lime are also weighed at the beginning and at the end of the experiment. Bottle *I* absorbs the water vapor, which permits the estimation of the latent heat of evaporation. Bottle *II* absorbs  $\text{CO}_2$  but liberates water in the reaction:



For that reason Bottle *III* is used to catch any water which may be lost from Bottle *II*. Therefore the increase in weight of Bottles *II* and *III* give the amount of  $\text{CO}_2$  liberated by the body. It is evident that the air entering the chamber has been freed of  $\text{CO}_2$  and moisture and has had its  $\text{O}_2$  content restored. The chamber may have facilities for the subject or animal to do work, such as a bicycle ergometer for a man or a treadmill for an animal, with suitable devices for measuring the work done. Thermometric measurements of the air and of the body temperature are likewise arranged for, and samples of air can also be obtained for analysis.

Using such an apparatus, Rubner, about the year 1894, showed, for a dog, that the caloric equivalent of the food taken in was equal to the heat output plus the heat equivalent of the urine and the feces. The error was within 1 per cent. He also showed that the direct method checked the indirect method; that is, the heat production calculated from the gas exchange. Later, Atwater and Benedict brought out the same facts using men as subjects. For example, the average results obtained for three men who were tested for forty days each were as follows:

	AVERAGE CALORIES PER DAY
Direct calorimetry	2,723
Indirect calorimetry	2,717
Difference	6, or 0.2 per cent

### The Respiratory Quotient

The respiratory quotient has an important bearing upon many phases of energy metabolism, whether we are dealing with the intact animal or with tissue preparations. It is the ratio of the volume of  $\text{CO}_2$  produced, to the volume of  $\text{O}_2$  used.

$$\text{R.Q.} = \frac{\text{Vol. CO}_2}{\text{Vol. O}_2}$$

For complete combustion of carbohydrates, the respiratory quotient is 1.0, as is seen from the following equation in which glucose is acted upon.



From Avogadro's law it is known that a given volume, at constant temperature and pressure, will contain the same number of molecules of *any* gas. Therefore, since six molecules of  $\text{CO}_2$  occupy the same volume as six molecules of  $\text{O}_2$ ,

$$\text{R.Q. of C}_6\text{H}_{12}\text{O}_6 = \frac{6 \text{ CO}_2}{6 \text{ O}_2} = 1.0.$$

The equation for a typical fat oxidation is as follows:



$$\text{R.Q., Triolein} = \frac{57 \text{ CO}_2}{80 \text{ O}_2} = 0.71$$

The other fats have slightly different respiratory quotients but all are about 0.7.

The respiratory quotient of protein is more difficult to determine since the protein molecule is not completely burned. It has been estimated by Loewy in the following manner. One hundred grams of meat protein contain:

52.38 Gm. C  
7.27 Gm. H  
22.68 Gm. O  
16.65 Gm. N  
1.02 Gm. S

After ingestion there are excreted in the urine and feces all of the nitrogen and sulfur, and part of the hydrogen, oxygen, and carbon. The amounts of C, H, and O not excreted in the urine and feces are:

41.50 Gm. C  
4.40 Gm. H  
7.69 Gm. O

This, then, is available for oxidation processes. The 7.69 Gm. of oxygen is sufficient to oxidize 0.96 Gm. of hydrogen, leaving for further oxidation and therefore for the computation of the respiratory quotient:

41.50 Gm. C  
3.44 Gm. H

The oxygen necessary for these oxidations would be 138.18 Gm., or 92.63 liters (138.18 Gm.  $\times$  0.699 liters, since 1 Gm. of oxygen occupies 0.699 liters of space). The carbon dioxide produced would be 152.17 Gm. or 77.39 liters (152.17 Gm.  $\times$  0.5087 liters, because each gram of carbon dioxide occupies 0.5087 liters). The respiratory quotient of the proteins is, accordingly,

$$\text{R.Q. protein} = \frac{77.39 \text{ l. CO}_2}{92.63 \text{ l. O}_2} = 0.801.$$

In general, 0.8 is used for the respiratory quotient of proteins.

If an animal could oxidize exclusively one food at a time, it is evident that when using carbohydrate his respiratory quotient would be 1.0, when burning fat it would be 0.7, and during protein utilization it would be about 0.8. Since this is not the case, and all three types of food are being metabolized simultaneously, the respiratory quotient is always a resultant of all types of metabolism. On an ordinary mixed diet the respiratory quotient is usually found to be about 0.85 for a normal individual. In the postabsorptive state, that is, some twelve hours after the last meal, it is usually slightly lower, about 0.82. However, the feeding of foods high in any one of the three chief foodstuffs can be found to have a definite influence on the respiratory quotient. Thus, with a respiratory quotient of 0.85, the feeding of carbohydrate will tend to raise it, and the feeding of fat, to lower it.

Since the three types of foodstuffs, with different caloric values, each have different R.Q.'s, it follows that the caloric value of one liter of oxygen absorbed will depend upon the particular foodstuff it is oxidizing. The same is true of the carbon dioxide produced. For example:



That is, 134 liters of oxygen are required to oxidize 180 Gm. of glucose or to produce  $180 \times 3.8$  Calories, since each gram of glucose will produce 3.8 Calories of heat. Therefore, with an R.Q. of 1, one liter of oxygen is equivalent to  $\frac{180 \times 3.8}{134}$  Cal., or about 5.1 Cal. Similarly, for a fat:



That is, 1,792 liters of oxygen are required for 884 Gm. of triolein or to produce  $884 \times 9$  Cal. Therefore, with an R.Q. of 0.7, one liter of oxygen is equivalent to  $\frac{884 \times 9}{1,792} = 4.4$  Cal. Therefore, if a person has a respiratory quotient of 1.0, it can be assumed not only that carbohydrate alone is burning, but if the volume of oxygen consumed in a definite period of time could be ascertained, the amount of carbohydrate burned in that time could be calculated. If the respiratory quotient were 0.7, the R.Q. of fat, how much fat was being consumed could similarly be ascertained. Since the R.Q. for protein is between these two figures, i.e., about 0.8, exclusive protein combustion cannot be assumed, since the simultaneous combustion of carbohydrate and fat and protein would result in an R.Q. of about this value.

As stated before, however, all three foodstuffs usually are being utilized at the same time. Nevertheless, calculations are possible which will give us the amounts of protein, carbohydrate, and fat being consumed at the same time. The protein catabolized during the experimental period can easily be found by obtaining the urine excreted during this time. The total nitrogen present is, of course, about 16 per cent of the protein catabolized, or

$$\frac{\text{Total N}}{0.16} \text{ or } \text{Total N} \times 6.25 = \text{Protein.}$$

The volumes of  $\text{CO}_2$  and  $\text{O}_2$  which the protein represents can be calculated and subtracted from the total  $\text{CO}_2$  and  $\text{O}_2$ . The remainder is the nonprotein  $\text{CO}_2$  and  $\text{O}_2$  from which the nonprotein R.Q. can be calculated. Now, a nonprotein R.Q. must vary between 0.7, the R.Q. for 100 per cent fat and no carbohydrate consumption, and 1.0, the R.Q. for 100 per cent carbohydrate and no fat catabolism. All R.Q. figures between 0.7 and 1.0 will indicate that a mixture of the two is being burned. Tables are available which give the amounts of each of these two constituents being burned (per liter of oxygen), for R.Q.'s between 0.7 and 1.0, and the number of calories that one liter of oxygen represents in this combustion. Thus, knowing the total N output, the  $\text{O}_2$  consumption and



the  $\text{CO}_2$  output in a given period, the number of grams of protein, carbohydrate, and fat catabolized can be estimated.

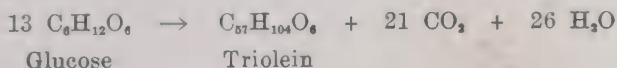
In metabolism experiments it is desirable to calculate the heat production from the oxygen consumption. As has been seen, this would be easy to do rather accurately if only carbohydrate and fat were being utilized. The protein makes it more difficult. However, extreme accuracy is not necessary in the indirect methods of calorimetry employing this calculation. At a respiratory quotient of 0.71 the number of Calories produced per liter of oxygen is only about 6 per cent less than that produced at a respiratory quotient of 1.0. These values are shown in Table XLII. For this reason the caloric value for oxygen consumption is usually based on the R.Q. observed, or, if it is not determined, "standard" R.Q. is assumed.

TABLE XLII  
ANALYSIS OF THE OXIDATION OF MIXTURES OF CARBOHYDRATE AND FAT\*

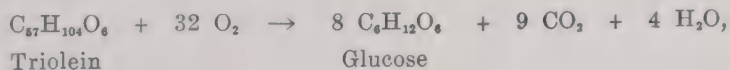
R. Q.	PERCENTAGE OF TOTAL OXYGEN CONSUMED BY		PERCENTAGE OF HEAT PRODUCED BY		CALORIES PER LITER OF $\text{O}_2$
	CARBOHYDRATE	FAT	CARBOHYDRATE	FAT	
0.707	0	100.0	0	100.0	4.686
0.75	14.7	85.3	15.6	84.4	4.739
0.80	31.7	68.3	33.4	66.6	4.801
0.82	38.6	61.4	40.3	59.7	4.825
0.85	48.8	51.2	50.7	49.3	4.862
0.90	65.9	34.1	67.5	32.5	4.924
0.95	82.9	17.1	84.0	16.0	4.985
1.00	100.0	0	100.0	0	5.047

\*From Lusk, G.: J. Biol. Chem. 59: 41, 1924 (abbreviated).

It should be mentioned that the respiratory quotient cannot be considered as the index of a single process. If carbohydrate is being converted to fat, this reaction by itself would result in a respiratory quotient greater than 1.0, because  $\text{CO}_2$  is produced and no  $\text{O}_2$  is used.



On the other hand, if fat were being transformed into carbohydrate, the R.Q. would be very low. For example, from this reaction,



One would have an R.Q. of about 0.28. The first of these reactions is accepted by all, and the second is claimed to occur by many authorities. The theoretical R.Q. for the conversion of protein to carbohydrate has been calculated as 0.6 to 0.7. There may be still other reactions which influence this value, including the fixation of  $\text{CO}_2$ , and physiological and pathological factors which modify it. Therefore it is apparent that the total R.Q. will be a figure representing a composite of the respiratory exchanges involved in all metabolic reactions. Nevertheless, since the oxidative processes predominate in the body over the synthetic ones just mentioned, the use of the R.Q. in studying heat production is justified.

### Metabolism of Ethyl Alcohol

Alcohol in moderate amounts is 90 to 95 per cent utilized. The rate of utilization varies somewhat with the individual but not with the quantity in-

gested; that is, the rate of oxidation is fairly constant. From 3.5 to 15 ml. of pure alcohol may be consumed by the organism per hour. The R.Q. of ethyl alcohol is 0.67 and the caloric value is 7 Calories per gram. Assuming an average utilization of 10 ml. or 8 Gm. per hour, this would amount to 56 Calories per hour. It is apparent that an individual could obtain an appreciable proportion of his caloric needs from alcohol alone.

The oxidation of alcohol seems to occur in the liver and insulin is necessary for its breakdown. In fact, treatment with insulin and glucose hastens recovery from ethyl alcohol intoxication. The products of alcohol catabolism are  $\text{CO}_2$  and water. Since alcohol cannot be stored in the body but can be converted into the energy of heat and work, it spares carbohydrate and fat and thus may increase glycogen and fat deposition. It may even become a protein sparer if the diet is deficient in carbohydrate. The small amount of alcohol which escapes oxidation is eliminated mainly through the lungs and kidneys.

### BASAL METABOLISM

There are several factors which contribute to the total heat production of the body. The principal ones are derived from the metabolism of the body at rest, the heat produced by work or exercise, and that due to the specific effects of food. In addition, a low atmospheric temperature will stimulate heat production, and sleep will depress it. Emotions, noises, and discomforts usually increase heat production also. The metabolism of the body at rest is called "basal metabolism." More exactly, basal metabolism, or the "basal metabolic rate," is defined as the heat production of the body when in a state of complete mental and physical rest and in the postabsorptive state.

The basal metabolic rate is frequently determined clinically. Since food, exercise, sleep, and external temperature all modify heat production, it is essential that these factors be excluded. Therefore, the subject is required to take the test after a twelve-hour fast; i.e., in the postabsorptive state. He is made warm and comfortable in a room which is quiet and which has subdued lighting. He must be informed about what is to be done so that he will not be alarmed, and in every possible way put in a resting condition. Under such conditions his metabolism is considered basal.

The heat production in the basal state may be determined directly by an Atwater-Rosa-Benedict calorimeter, and this is the most accurate procedure. Since this is not feasible ordinarily, indirect methods have been devised, based upon the principles discussed. By the indirect methods, either the oxygen consumption and carbon dioxide output are determined, or only the oxygen consumption. In the former instance the R.Q. can be determined and this, as has been seen, permits us to get a more exact idea of the calorific value of the oxygen being consumed. If only the oxygen consumption is determined, the R.Q. must be assumed to be the average value usually found. In general, then, the basal metabolic rate (B.M.R.) for a given period is obtained by multiplying the volume of oxygen consumed during that period by the calorific value for oxygen corresponding to the observed (*or assumed*) respiratory quotient.

There are, in general, two systems available for the indirect determination of the B.M.R. These are the "open circuit system," in which both the oxygen consumption and carbon dioxide output are measured, and the "closed circuit system," in which only the oxygen consumption is estimated. Both can be used clinically, but the former requires a high degree of technical skill, more cumbersome apparatus, and is less rapid. Since it is the more accurate, it will be described first.

**Open Circuit Systems.**—The Tissot method and the Douglas method are both open circuit systems. The subject breathes through a valve system, which is so arranged that he inspires pure atmospheric air and his expired air is collected either in a Tissot spirometer or a Douglas bag. The latter is a rubber

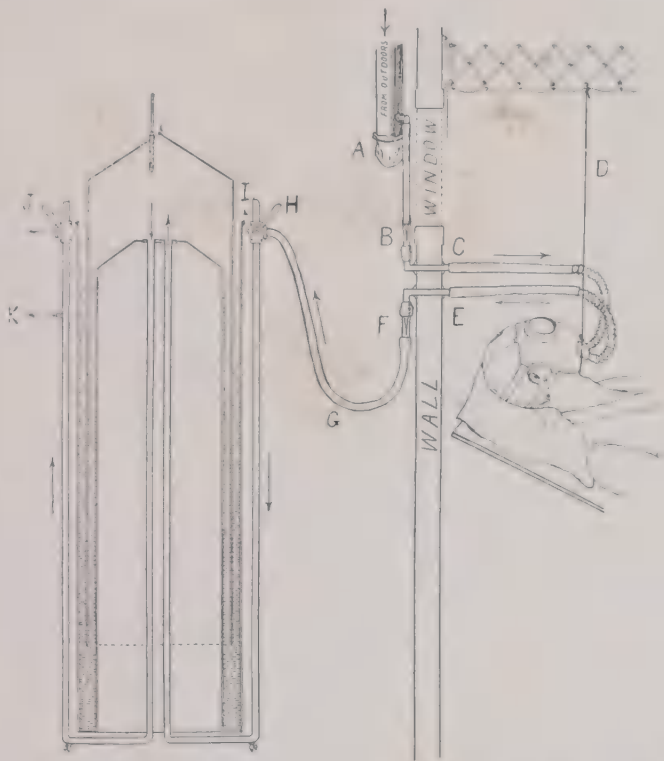


Fig. 76.—Apparatus for the determination of the basal metabolism by the open circuit method. The subject lying at rest on a cot and wearing a face mask is separated by a wall and a window from the spirometer. *C* and *E* are pipes leading from the mask to outside air and inspiratory air to spirometer from mask. The spirometer consists of a bell which is arranged to rise freely as the expired air is collected under it. The entire dead space of the apparatus can be flushed out with expired air by allowing it to escape through the valve. On closing this valve the expired air is caught in the spirometer bell. *H* is a valve which permits expired air to escape through *I*, a vent. (From Bailey, C. V.: *J. Lab. & Clin. Med.* 657, 1921.)

of 60 to 100 liters capacity, which has the advantage of being portable, and is also useful in energy experiments involving exercise. The spirometer, on the other hand, is easily calibrated so that the volume of expired air can be accurately observed on the instrument itself. The passage of the air may be accomplished by having the subject breathe through a mouthpiece connected with the apparatus. In this case a clip prevents nose breathing. A more comfortable arrangement is the use of a mask of special design which can be made



airtight. The valves used to ensure the passage of air in the right direction must have low resistance so that little extra work is done by the subject in overcoming this resistance. Fig. 76 is a diagram showing a Tissot, or gasometer, apparatus in use. The subject inspires fresh air and expires into the spirometer, or gasometer. After a given period of time the volume of expired air is estimated and a sample is analysed. The composition of inspired air is also determined by analysis. However, the *volume* of inspired air is not measured. It can be calculated from the percentages of nitrogen in both the inspired and expired air, since nitrogen is an inert gas and the *total volumes* of nitrogen inspired and expired must be equal. It is thus possible to determine the volumes of oxygen utilized and carbon dioxide produced, having given the percentages of  $\text{CO}_2$  and  $\text{O}_2$  in both the inspired and expired air, and the volume of expired air. Consequently the R.Q. is readily found. Since each liter of oxygen retained corresponds to a certain number of calories of heat for the respiratory quotient in question, it is a simple matter to calculate the number of calories produced during the experimental period.

**Closed Circuit System.**—In these methods, which are the more commonly used clinical procedures, the passage of air may be controlled either by a mouth-piece or a mask, as in the open circuit systems. However, here fresh air is not continually inspired. The system is filled with oxygen, and any diminution in the total volume is due to oxygen consumption because  $\text{CO}_2$  and  $\text{H}_2\text{O}$  from the lungs are absorbed by soda lime as fast as they are formed. It is a closed system, and the respired gas is breathed over and over again. The Benedict-Roth apparatus is shown in diagrammatic form in Fig. 77. There is one respiration valve in the inspiration tube and a second one at the outlet of the  $\text{CO}_2$  and  $\text{H}_2\text{O}$  absorber. These direct the air in the right path. There is a spirometer bell suspended by a cord which passes over a pulley and is balanced by a counterweight. With each respiration the spirometer rises and falls and a pointer on the counterweight writes on a kymograph a record of this movement. Before starting the test the spirometer is emptied of gas and is then filled with oxygen. As the subject breathes, he retains some of this oxygen and expires a mixture of  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{O}_2$ , and  $\text{N}_2$ . The  $\text{CO}_2$  and  $\text{H}_2\text{O}$  are absorbed by the soda lime, and the spirometer will gradually fall as the oxygen is used up. The slope of the curve is used to measure the oxygen consumption in a six-minute period.

Since the  $\text{CO}_2$  is not measured in this method, an R.Q. of 0.82 is assumed. This gives a heat value for each liter of oxygen consumed of 4.825 Cal. (see Table XL). The spirometer bell is designed to have a volume of 20.73 ml. for every millimeter of height. Therefore each millimeter which the spirometer falls in six minutes is equivalent to  $\frac{20.73}{1000}$  liter  $\times$  4.825 Cal., or 0.1 Cal. in six minutes, or 1 Cal. per hour. The kymograph paper is ruled in millimeters so that the heat production can be directly obtained by observation. Furthermore, timing is rendered unnecessary by having vertical lines spaced at intervals, each equivalent to one minute. Fig. 69 shows an oxygen line drawn in relation to the "Calorie" and "Time" lines. Corrections for temperature and barometric pressure must, of course, be made, but these and all calculations are explained on the back of the tracing paper.

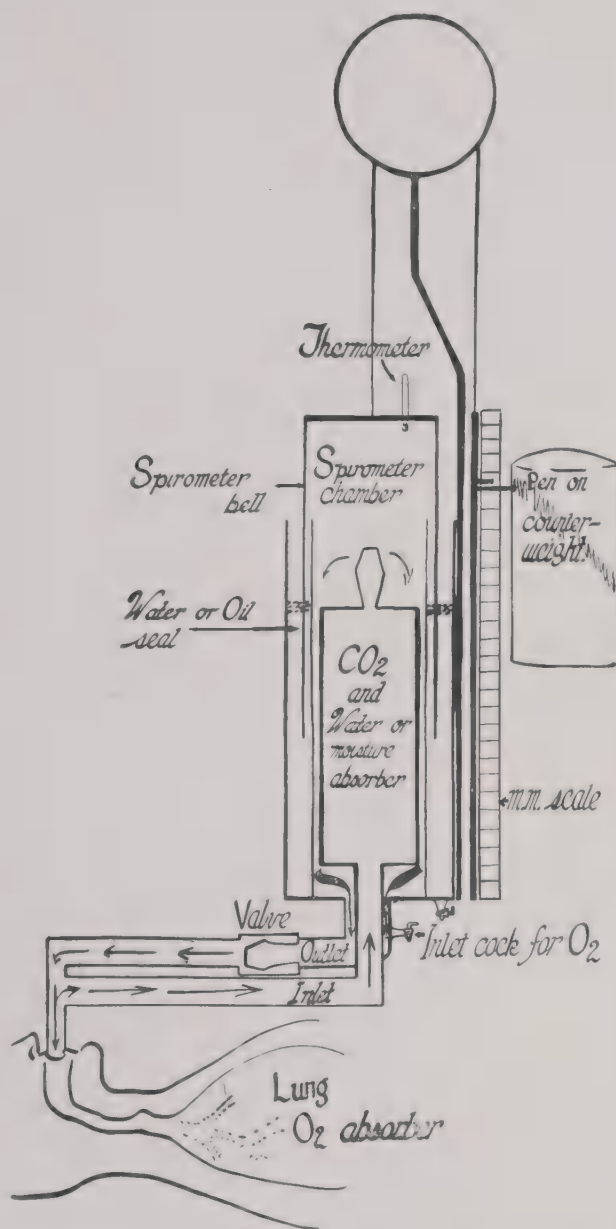


Fig. 77.—Diagram of Benedict-Roth apparatus for the determination of basal metabolism. The patient breathes through a mouthpiece which fits between the gums and the lips; the mouthpiece is clamped. Two tubes connect the mouthpiece with the spirometer, and appropriate valves permit the air to flow only in the direction indicated. The expired air passes through a lime which absorbs carbon dioxide and water. The calibrated spirometer bell is balanced by a counterweight carrying a pen, which writes the record on a millimeter scale. The spirometer chamber is filled with oxygen when the test starts. As oxygen is absorbed by the subject, the volume of the gas in the chamber decreases and this decrease in volume is used as the basis for calculations.

(Modified from Roth, P.: Boston M. & S. J. 186: 491, 1922.)

**CALCULATING THE BASAL METABOLIC RATE.**—Let it be assumed that the oxygen line, a straight line drawn through a majority of the lower peaks of the curve, intersects a vertical "minute line" at 58 mm. Another intersection six spaces from the first one, is, say, at 128 mm. (see Fig. 78). The oxygen consumption, then, in six minutes is represented by

$$\begin{array}{r} 128 \\ - 58 \\ \hline 70 \text{ mm.} \end{array}$$

Since each millimeter for six minutes is equivalent to one calorie per hour, the heat output is 70 Cal. per hour. This must now be corrected for temperature and pressure. Assuming 20° C. and 750 mm., it is found from a table (on the back of the tracing paper) the corresponding factor 0.902.

$$70 \times 0.902 = 63.1 \text{ Cal. per hour}$$

The corrected figure is 63.1 Cal. per hour.

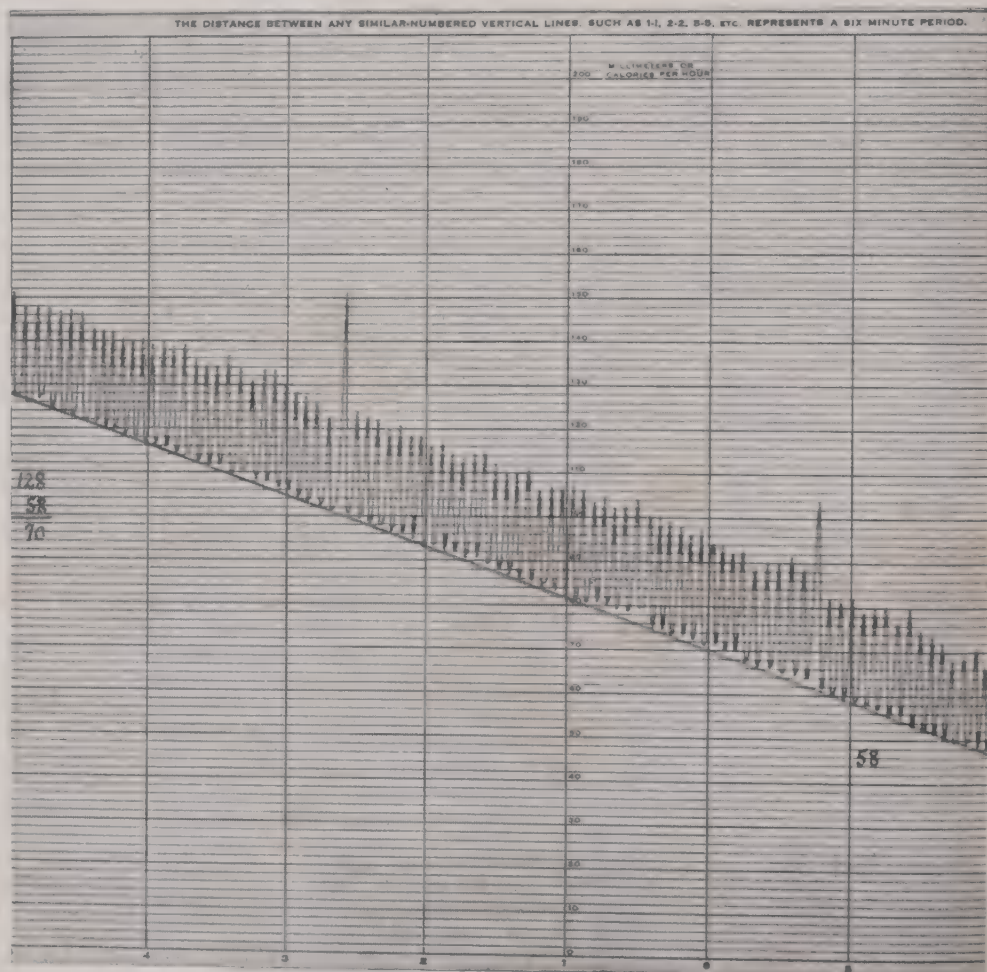
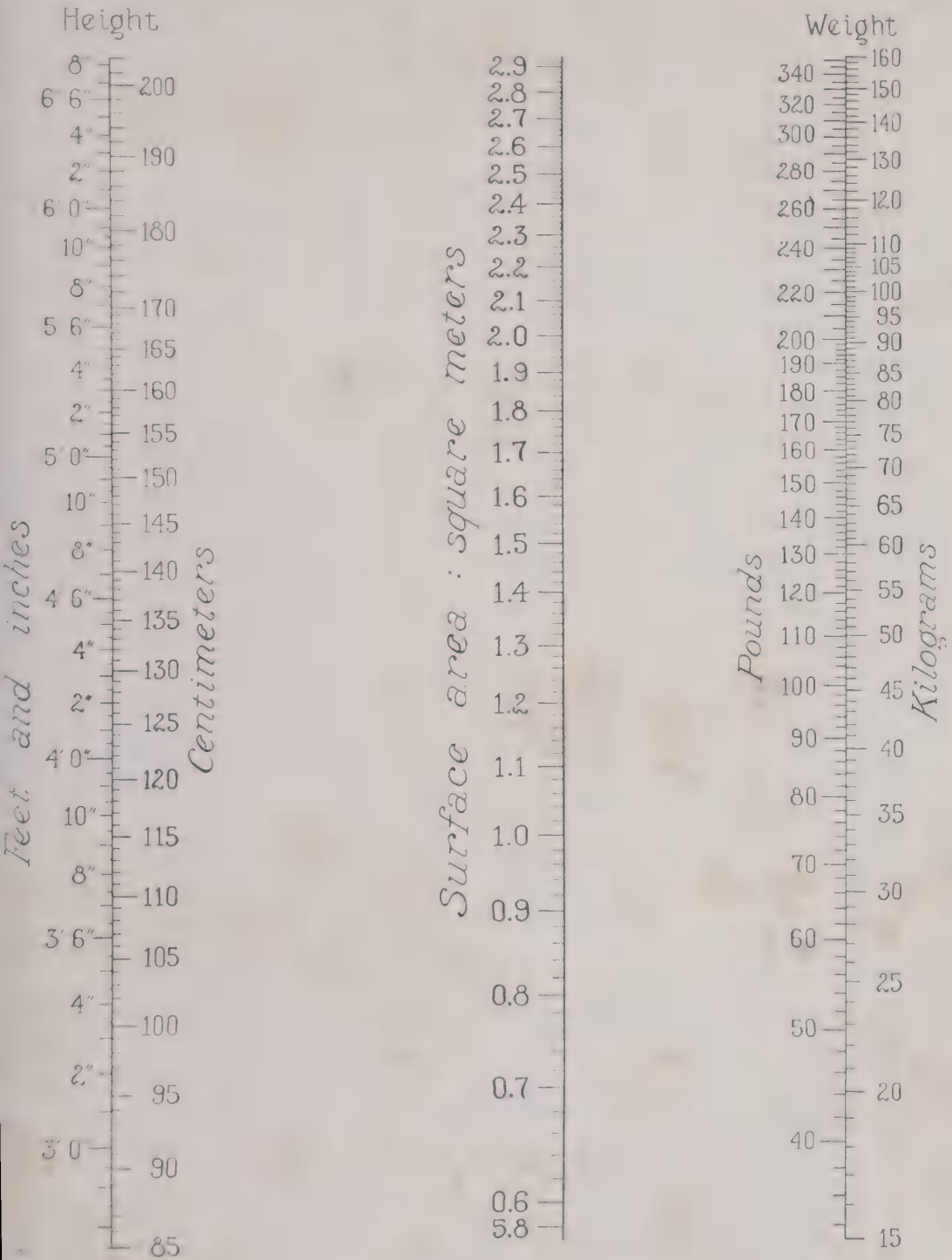


Fig. 78.—A typical oxygen line in a basal metabolism test using a Benedict-Roth apparatus. (Courtesy Warren E. Collins, Inc., Boston, Mass.)

This must now be related to the normal values. If the subject is a woman, aged 50 years, 5 feet, 4 inches tall, and weighing 108 pounds, it may be found from Table XLII that her output should be 34.0 Cal. per square meter surface area per hour. From the nomogram (Fig. 79), a line drawn between the points indicating her height and weight crosses the middle line at 1.50 square meters of surface area. Therefore, the normal B.M.R. would be

$$34.0 \text{ Cal. per square meter} \times 1.5 \text{ square meters} = 51.0 \text{ Cal.}$$





### Surface Area by formula of DuBois

Fig. 79.—Nomogram for estimating surface area from weight and height, according to DuBois' formula. A straight line drawn from a point corresponding to the height of the individual on the left-hand scale to that of his weight on the right-hand scale crosses the middle line at a point indicating his surface area. (Courtesy Dr. W. M. Boothby.)

This is now subtracted from the figure (corrected) found,

$$63.1 - 51.0 = 12.1 \text{ Cal. per hour above normal,}$$

and this excess divided by the normal; i.e.,  $12.1/51.0 = +24\%$ ; that is, in this instance the B.M.R. is 24 per cent above the normal for a woman of the age, height, and weight given.

### Normal Influences

From the data obtained by any of the methods outlined, the basal metabolic rate, that is, the caloric output at rest, is determined. There are several factors which normally influence this. Consequently, in order to ascertain whether the B.M.R. determined is normal or not, it is necessary to compare it with normal standards. (All these factors have been taken into consideration in the calculations of the hypothetical test described above.)

**Influence of Size.**—It is of course true that the greater the size of an animal, the greater will be the total heat production. But the amount of heat produced is not proportional to the body *weight*. In 1901 Voit showed that it bore a much closer relationship to *surface area* than to weight and, shortly after, Rubner proposed the law that total metabolism is proportional to the superficial area of an animal. This is clearly evident from Table XLIII. This

TABLE XLIII  
RELATION OF HEAT PRODUCTION TO BODY WEIGHT AND SURFACE AREA\*

	WEIGHT IN KILOGRAMS	CALORIES IN 24 HOURS	
		PER KILOGRAM OF BODY WEIGHT	PER SQUARE METER OF BODY SURFACE
Pig	128	19	1,078
Man	64	32	1,042
Dog	15	52	1,039
Mouse	0.018	212	1,188

\*From Rubner.

law holds not only for different species, but also for individuals of the same species of different sizes. Thus a small man will have a greater heat output than a large one per pound, but when calculated to their surface areas the heat output will be about the same. For this reason, the B.M.R. is either expressed as Calories per square meter of surface area or the total number of Calories produced is compared with that which would be produced by a normal individual having the same surface area.

The surface area of a man is difficult to estimate accurately. Many measurements have been made, and there has been established a fairly constant relationship between the body weight and height. This is expressed by the formula of DuBois and DuBois:

$$\text{Surface area in sq. cm.} = (\text{Weight in kg.})^{0.425} \times (\text{Height in cm.})^{0.725} \times 71.84$$

A graphic method of obtaining the same result is by the use of a "nomogram," shown in Fig. 79 (Boothby and Sandiford).

Recent work indicates that although there is a considerable correlation between metabolic rate and body surface, it is not quite so definite as is in-

licated by Rubner's "law." The figures found today for total daily heat production per square meter of body surface do not quite agree with those found by Rubner (Table XLIII), and authorities in this field have suggested a power function of body weight as a standard instead of the surface area. Thus Brody adopts  $W^{0.7}$  as the reference base,  $W$  representing body weight in kilograms. Kleiber maintains that  $W^{3/4}$  is more accurate. Because surface area for all animals is approximately equivalent to  $W^{2/3}$ , a value not far from those just given, the clinical measurements based on the DuBois formula or nomogram has been found to be moderately accurate for clinical purposes.

**Influence of Age and Sex.**—At birth the basal metabolic rate is said to be very low, but after the infant begins to gain weight the B.M.R. increases rapidly and it is very high for the first three to six years. Thereafter the heat production diminishes with increasing age and the decrease is very gradual indeed during adult life. These facts have been obtained by thousands of determinations by the indirect method. As a result we have data which show approximately the normal B.M.R. for any age and for either sex. Females have from 2 to 12 per cent lower rates than males; adults from 10 to 12 per cent lower than children. The relationship of sex and age are shown in Table XLIV. These "normal standards" are occasionally revised as more determinations under carefully controlled conditions are obtained.

TABLE XLIV

## RELATIONSHIP OF AGE AND SEX TO METABOLISM

(THE DUBOIS NORMAL STANDARDS AS MODIFIED BY BOOTHBY, BERKSON, AND DUNN)\*  
CALORIES PER SQUARE METER PER HOUR

AGE (YR.)	MALES	FEMALES	AGE (YR.)	MALES	FEMALES
6	53.0	50.6	20-24	41.0	36.1
7	52.4	49.1	25-29	40.0	35.9
8	51.8	47.0	30-34	39.3	35.8
9	50.5	46.1	35-39	38.7	35.6
10	48.5	45.7	40-44	38.0	35.5
11	47.2	45.3	45-49	37.4	34.9
12	46.8	44.3	50-54	36.7	34.0
13	46.5	42.9	55-59	36.1	33.2
14	46.4	41.4	60-64	35.5	32.7
15	46.1	40.1	65-69	34.9	32.3
16	45.7	38.8	70-74	34.1	32.1
17	44.8	37.8			
18	43.3	37.0			
19	42.3	36.6			

\*From data in Am. J. Physiol. 116: 468, 1936.

**Other Physiological Factors.**—The data mentioned were obtained in the United States on individuals of the various races which make up our population. However, there may be some variation among races. Natives of Yucatan have been found to have generally a higher metabolic rate than ours, while some Chinese women living in America had a lower rate. White individuals usually show a decreased metabolism when they live in tropical countries.

The metabolism of women fluctuates much more than that of men. Before menstruation the rate usually rises and after menstruation it falls. These



facts must be taken into consideration when studying the results of tests. It has also been shown that although ordinarily the B.M.R. of women is from 10 to 12 per cent below that of men, in a very warm environment it may be from 15 to 20 per cent below that of men, and in a cold climate it may be the same as in men.

Normal pregnancy has little influence upon the B.M.R., although, of course, the total amount of heat produced will be the sum of that produced by the mother and the fetus. Athletes and laborers usually have a somewhat higher rate than other people because of a greater degree of muscular development.

The determination of the B.M.R. in persons who are ill is best made in the same building where they spend the night. The excitement and disturbance of even a short ride is sufficient to increase the B.M.R. up to as much as 50 per cent. On the other hand, a person in good health may travel for an hour or more, rest for a half hour, and be in "basal condition." The novelty of the experience may also be a disturbing factor. Unless the operator understands how to reassure the patient, a first B.M.R. test is likely to be from 5 to 10 per cent too high.

### Pathological Influences

The most important practical use for B.M.R. determinations is in the diagnosis and treatment of thyroid conditions. The hormone elaborated and secreted by the thyroid has the property of stimulating the metabolic activities of the cells. A hypersecretion, therefore, causes an increased rate of basal metabolism. A hyposecretion, on the other hand, results in a lowered B.M.R. Before thyroid operations for hyperthyroidism, before administering the hormone for hypothyroidism, and in following the effects of operations or treatment, B.M.R. determinations are invaluable. Too much emphasis cannot be laid upon the necessity for careful technical work in performing these tests. If the operator does not comprehend the basis for the determination, he is likely to get completely erroneous results, either because the patient is not in a state of complete rest or because of lack of technical knowledge or skill.

Fevers raise the metabolic rate. The B.M.R. increases by about 5 per cent for each degree Fahrenheit above the normal body temperature. The reason for this is evident, since an increased body temperature is primarily due to increased cellular activity. It is therefore imperative when doing "basals" to take the temperature of the patient. If he has a subnormal temperature, he should be given additional covers, hot pads, etc., to bring his temperature to normal.

Since the adrenal gland secretes a hormone, adrenaline, which also increases cellular activity, affections of this gland may also change the B.M.R. Adrenaline's action, however, is ordinarily fleeting and this may account for the temporarily high rates which sometimes are encountered in nervous patients. This is also one of the reasons for requiring mental as well as physical repose when determining the B.M.R., since the emotional excitement causes increased secretion of adrenaline. In Addison's disease, a condition in which the adrenal

cortex is damaged, the B.M.R. is low. This is probably not due to a lack of adrenaline, which is produced in the adrenal medulla, but to a deficiency of the adrenal cortical hormone.

In the vast majority of cases, the B.M.R. is within normal limits. These are generally assumed to be from 10 per cent above to 10 per cent below the normal standard for the age, sex, and surface area of the individual studied. The pathological variations mentioned may have rates varying from 40 per cent below to 130 per cent above the average normal. Besides thyroid disorders, fevers, and Addison's disease there are a few other pathological conditions in which the basal metabolic rate may be altered. The complete list is as follows, but it must be remembered that the effect on the B.M.R., except in the instances previously noted, is not invariable.

#### BASAL METABOLIC RATE BELOW NORMAL

Hypothyroidism

Addison's disease and other types of hypoadrenalism

Starvation and malnutrition

Hypopituitarism

Lipoid nephrosis

Shock

#### BASAL METABOLIC RATE ABOVE NORMAL

Hyperthyroidism

Cushing's syndrome (Basophilic adenoma of the pituitary)

Tumors of the adrenal gland

Fever

Leukemia

Polycythemia

Anemia

Essential hypertension

Myocardial insufficiency

Diabetes insipidus

**Effect of Thiouracil on the Basal Metabolic Rate.**—The specific action of thiouracil and related compounds upon the thyroid gland will be discussed in Chapter 23. It may be pointed out here, however, that this action results in a reduction of the basal metabolic rate. Hence, any pathological effect due to a high B.M.R. may be treated by using this drug, provided the underlying cause is a hyperthyroidism. The opposite effect, i.e., an increase in the B.M.R., is produced by the administration of thyroxine or related substances (page 615). This may also be accomplished by certain drugs, notably dinitrophenol.

**Obesity.**—The deposition of excess fat in the body has led to many fallacious ideas on the subject. One frequently is told that a certain stout person eats very little, that it is his "nature" to be fat, that he has an endocrine condition, etc. There is no way of avoiding the fact that the law of conservation of matter and energy holds for the fat as well as for the thin. If intake of food exceeds output of the equivalent amount of heat and energy, fat must be stored (as well as glycogen and protein). The only exceptions are those conditions in which the food is not digested or absorbed properly. Such a state of affairs may account for the lack of adiposity which some heavy eaters exhibit.

But if food is normally digested and absorbed and the calories ingested equal the total calories expended, there will be no appreciable change in weight. Why, then, is there a tendency to "put on weight" with increasing age? The answer is that the basal metabolic rate decreases as one gets older. Less food is needed, but the food habit or appetite remains. There is also the tendency to exercise less. Hence, less of the food intake is utilized and it is therefore stored chiefly as fat.

Another puzzling fact is the inability to lose weight frequently experienced by obese individuals when they go on a reducing diet. This has been explained by Newburgh in careful water balance experiments. Obese patients on a reducing diet frequently do not lose weight for as long as two weeks. Sometimes they even gain weight. This is due to a retention of water by the tissues. After a time, however, the water is eliminated and the weight drops. Fig. 80 shows a typical graph.

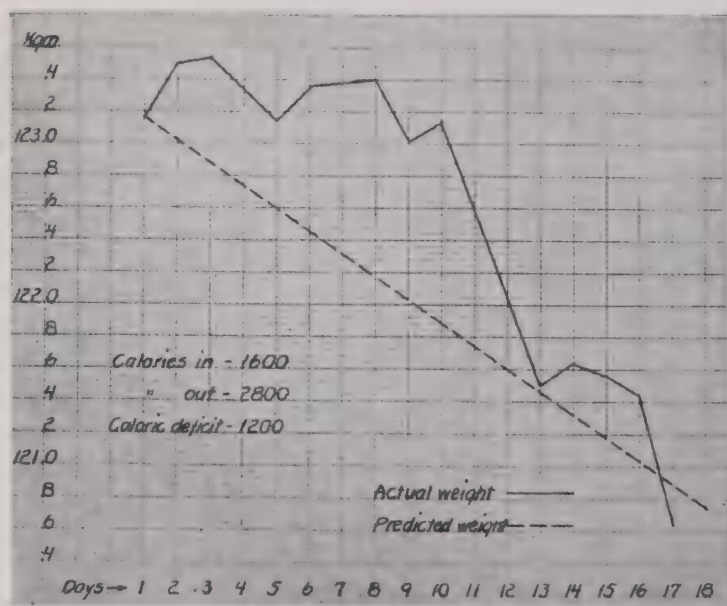


Fig. 80.—Weight chart of an obese patient on a reducing diet. The increases in weight and failure to follow the predicted weight line are ascribed to addition and retention of water by the tissues. (From Newburgh, L. H.: *Physiological Rev.* 24: 18, 1944.)

Several endocrine conditions, other than those already mentioned, are commonly believed to be associated with a pathological state of obesity. This can only mean that they influence the appetite, since there are very few cases of abnormally low B.M.R. among them. Indeed an abnormally low metabolism is as common among thin as among fat people. However, abnormalities of the endocrine system do affect the *distribution* of adipose tissue and the conformation of the figure can thus aid in diagnosing the type of endocrine dysfunction (Conn).

### Specific Dynamic Action of Foods

If a person's basal metabolic rate is known, one would expect that the ingestion of the amount of food corresponding to this value would result in the



production of this same quantity of heat, provided the subject remained at rest. The fact is that a greater amount of heat is put out than is represented by the calories ingested. For example, if the basal metabolic output is 1,800 Cal. in twenty-four hours, or 900 Cal. in twelve hours, the ingestion of food equivalent to 900 Cal. will result in an output during the next twelve to eighteen hours of perhaps 950 Cal. To furnish this extra energy, food stores of the body must be drawn upon. This is called the "specific dynamic action" (S.D.A.) of foods. Protein has the greatest specific dynamic action, amounting to about 30 per cent; carbohydrate causes an increase of about 5 or 6 per cent; and fat about 4 per cent. Ordinarily the specific dynamic action of all together amounts to about 6 per cent of the basal metabolic rate. Recent experiments of Forbes and his associates emphasize the fact that the specific dynamic action of any combination of foodstuffs is not the sum of their individual values but is invariably less. Furthermore, when such mixtures are fed, protein does not dominate the specific dynamic action as was formerly believed. Fat seems to be more potent than either of the other two nutrients and apparently confers economy of utilization upon the food mixtures in which it occurs; i.e., it lowers the specific dynamic action (S.D.A.) to a greater extent than does either of the others.

The explanation for the specific dynamic action is not clear. It cannot be due to a production of heat as a result of digestion, because the feeding of the products of digestion are just as effective as the undigested substances. In fact, the intravenous injection of the amino acids gives rise to a specific dynamic effect of the same order as results from feeding. Recent studies indicate that the specific dynamic action of the various amino acids is best correlated with the metabolizable energy of the individual amino acid (Kriss). That is, it is not related to the nitrogen but rather to the nonnitrogenous fraction. This underlies oxidative and synthetic changes which liberate heat. In other words, this heat is evolved during the intermediary metabolism of the carbon chains. The S.D.A. of glucose is increased if thiamine is administered at the same time. Now, since thiamine stimulates the formation of fat from glucose, Ring concludes that the S.D.A. of glucose is due to the energy required to prepare it for deposition as fat. Possibly this is the explanation of the S.D.A. of all foodstuffs; i.e., the energy required to prepare the nonnitrogenous parts of the molecule for storage.

**Isodynamic Law.**—Rubner formulated a "law" to the effect that the different foodstuffs may replace each other in the diet for energy as well as for heat production in proportion to their calorific value; that is, a certain number of calories in a given food is equivalent to the same number of calories in any other food, regardless of the proportion of protein, carbohydrate, and fat. Although this is true in a general way, it must not be forgotten that proteins are in a class by themselves and must be provided in every diet in a suitable amount and of the proper quality. One cannot, therefore, substitute carbohydrate and fat for protein, even though their total caloric value is the amount needed. Nor would it be wise to make up a diet of protein and either carbohydrate or fat. Unusually large amounts of carbohydrate might lead to alimentary glycosuria, while an overabundance of fat in the diet might cause ketosis. A

mixture of the three is more physiological. Carbohydrate is a better protein sparer than fat and is usually the least expensive of the foodstuffs. It has recently been found that when the percentage of fat in a diet is varied, while maintaining equal quantities of energy and protein, the animals exhibit certain differences in nutritional behavior. For instance, with a higher fat diet there is a better economy of food utilization and an increased activity. (Forbes Black.) However, the concept of isodynamic equivalence is a very useful one from a dietetic standpoint. "One hundred Calorie portions" are commonly given in food tables or demonstrations and aid materially in computing diets.

### INFLUENCE OF MUSCULAR WORK UPON TOTAL METABOLISM

Muscular work is accomplished by the body at the expense of increased metabolism. The potential energy of the foodstuff is transformed into the free energy of work and the energy of heat. The latter, as has been seen, is greater than the former, and although the body is a good machine, as machines go, it is

TABLE XLV

ENERGY EXPENDITURE PER HOUR UNDER DIFFERENT CONDITIONS OF MUSCULAR ACTIVITY\*

FORM OF ACTIVITY	CALORIES PER HOUR		
	PER 70 KILOGRAMS	PER KILOGRAM	PER POUND
Sleeping	65	0.93	0.43
Awake lying still	77	1.10	0.50
Sitting at rest	100	1.43	0.65
Reading aloud	105	1.50	0.69
Standing relaxed	105	1.50	0.69
Hand sewing	111	1.59	0.72
Standing at attention	115	1.63	0.74
Knitting (23 stitches per minute on sweater)	116	1.66	0.75
Dressing and undressing	118	1.69	0.77
Singing	122	1.74	0.79
Tailoring	135	1.93	0.88
Typewriting rapidly	140	2.00	0.91
Ironing (with five-pound iron)	144	2.06	0.93
Dishwashing (plates, bowls, cups, and saucers)	144	2.06	0.93
Sweeping bare floor (38 strokes per minute)	169	2.41	1.09
Bookbinding	170	2.43	1.10
"Light exercise"	170	2.43	1.10
Shoemaking	180	2.57	1.17
Walking slowly (2.6 miles per hr.)	200	2.86	1.30
Carpentry, metalworking, industrial painting	240	3.43	1.56
"Active exercise"	290	4.14	1.88
Walking moderately fast (3.75 miles per hour)	300	4.28	1.95
Walking down stairs	364	5.20	2.36
Stoneworking	400	5.71	2.60
"Severe exercise"	450	6.43	2.92
Sawing wood	480	6.86	3.12
Swimming	500	7.14	3.25
Running (5.3 miles per hour)	570	8.14	3.70
"Very severe exercise"	600	8.57	3.90
Walking very fast (5.3 miles per hour)	650	9.28	4.22
Walking up stairs	1100	15.8	7.18

\*Compiled by M. S. Rose, from Sherman, H. C.: Chemistry of Food and Nutrition, ed. 6, New York, 1941, The Macmillan Co.

is quite uneconomical. Therefore, in order to perform the work, or exercise, the organism must have potential energy for the work and for the excess heat which is simultaneously liberated. A man sitting quietly has a total metabolism, on the average, of about 100 Cal. per hour. When he stands up, his metabolism increases by about 10 per cent because of the greater tonus of the muscles, and when he engages in active work it may increase to 300 Cal. or more per hour. The type of work, or exercise, will influence the total amount of energy output, heavy work requiring more energy than light. From Tables XLV and XLVI one can see how various types of activity affect the total energy expenditure. Table XLVII shows similar figures but in a different way; here the extra Calories, i. e., the amount *above* the basal rate, are shown, whereas in Tables XLV and XLVI the figures are for the total caloric output.

TABLE XLVI

TOTAL CALORIC REQUIREMENTS FOR TWENTY-FOUR HOURS\*

MALES	CAL.	FEMALES	CAL.
Shoemaker	2,000-2,400	Seamstress (needle)	1,800
Carpenter or Mason	2,700-3,200	Seamstress (machine)	1,900-2,100
Farmers	3,200-4,000	Household servants	2,300-2,900
Lumberman	5,000 or more	Laundress	2,600-3,400

\*Data from Tigerstedt and from Lusk.

It is thus easy to understand why different types of workers will liberate varying amounts of energy and will therefore require different amounts of calories in their diets.

### INFLUENCE OF MENTAL WORK UPON TOTAL METABOLISM

Mental work results in very little increase in total metabolism. Benedict found, for instance, that the effort involved in solving mathematical problems increased metabolism by only 3 or 4 per cent. This does not mean that the metabolism of brain is low. In fact, just the opposite is the case. Brain tissue has a high *basal* metabolism amounting to about one-tenth of that of the entire body, but the additional work which it performs in thinking does not result in much of an increase over this high basal figure.

### INFLUENCE OF SLEEP

During normal sleep the muscles are relaxed and the total metabolism correspondingly low. It is usually 10 per cent below the basal metabolic rate. In fact, if the metabolic rate could be determined routinely during sleep, this would be the true basal metabolic rate, since it is the physiological minimal rate. Since this is usually not possible, the conditions previously outlined are always observed.

### TOTAL HEAT PRODUCTION

All of the factors which go to make up the total heat production of an individual may now be listed. From such calculations figures like those in Table XLVI have been obtained. Take, for instance, the figures for a car-



penter. From a determination of the basal metabolic rate we get, say, 1,500 Cal. Then from Table XLVII it may be seen that he expends 164 Cal. per hour while working eight hours, and it may be assumed that he expends 74 Cal. per

Basal Metabolism—24 hr.	1,500 Cal.
8 hours sleep	-50 Cal. for sleep (10 per cent of basal for eight hours)
8 hours work	1,312 Cal. ( $8 \times 164$ )
8 hours sedentary or light exercise	592 Cal. ( $8 \times 74$ )
Specific dynamic factor	90 Cal. ( $1,500 \times 0.06$ )
	<hr/> 3,444 Cal.

hour (20 per cent above basal) during the remaining eight hours. During sleep there is a diminished heat production, and the specific dynamic factor adds about 6 per cent of the B.M.R.

Using the data in Table XLV, also for a man engaged in carpentry, we get:

8 hours sleep at 65 Cal.	520 Cal.
2 hours light exercise at 170 Cal.	340 Cal.
8 hours carpenter work at 240 Cal.	1,920 Cal.
6 hours sitting at rest at 100 Cal.	600 Cal.
Specific dynamic action	90 Cal.
Total	<hr/> 3,470 Cal.

The two hypothetical carpenters, therefore, have about the same total caloric output. In the first case we began with a basal output and added and subtracted the additional energy factors. In the second, the total caloric output per hour was tabulated plus the specific dynamic factor.

TABLE XLVII  
EXTRA CALORIES OF METABOLISM ATTRIBUTABLE TO OCCUPATION\*

	EXTRA CALORIES PER HOUR
Occupations of men	
Tailor	44
Bookbinder	81
Shoemaker	90
Metalworker, filing and hammering	141
Carpenter making a table	164
Stonemason chiseling a tombstone	300
Man sawing wood	378
Occupations of women	
Seamstress, needlework	6
Typist, fifty words per minute	24
Seamstress, using sewing machine	57
Housemaid, moderate work	81
Laundress, moderate work	124
Housemaid, hard work	157
Laundress, hard work	214

\*After Harrop.

## METABOLISM OF CHILDREN

The total metabolism in childhood is relatively much greater than in adult life. There is, in the first place, the high basal metabolic rate of childhood. In addition, the physical activity of children is usually greater, despite the fact that their period of sleep is longer than that of the adult. Their games and play involve a tremendous amount of muscular exercise. The food intake.

Therefore, must cover these caloric needs in addition to the extra food required for growth. A child of 12 years consequently needs about the same amount of food as an adult, while an active boy of 16 years requires 3,800 Cal. or more per day.

### PRACTICAL CONSIDERATIONS

Having in mind the various factors mentioned, it should be possible to calculate roughly the number of calories required by a given individual. The basal metabolic rate for normal persons can be estimated from Table XLIV, if the subject's height and weight are known. Although the normal person's B.M.R. may be up to 10 per cent above or below this "standard normal" value, and although the B.M.R. for a given individual is not constant from day to day, one can get a rough idea of that factor. According to duBois and Chambers, one may add 10 per cent to the B.M.R. to estimate the caloric requirement of a person quiet in bed; 30 per cent for a moderately active patient; and 50 per cent for a patient out of bed but indoors and moderately quiet. For activities of normal subjects Tables XLV, XLVI, or XLVII, may be used as mentioned previously.

The Food and Nutrition Board of the National Research Council has made recommendations, which are summarized in Table XLVIII (see also Table XIX, page 326).

TABLE XLVIII  
TOTAL CALORIC REQUIREMENTS

		CALORIES PER DAY	
		MALES	FEMALES
Moderately active	70 Kg.	3,000	56 Kg. 2,400
Very active	70 Kg.	4,500	56 Kg. 3,000
Sedentary	70 Kg.	2,400	56 Kg. 2,100
Children, up to 1 yr.		110 per kg.	110 per kg.
2 yr.		1,200	1,200
5 yr.		1,600	1,600
8 yr.		2,000	2,000
11 yr.		2,500	2,500
13-15 yr.		3,200	2,600
16-20 yr.		3,800	2,400

In normal people, appetite will generally regulate the intake of food so as to provide enough for caloric needs, replacement, growth, etc. However, in disease and obesity it is a poor guide indeed, and the physician and nutritionist must have in mind the basic principles in order that the approximate requirements of the individual are met.

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## Chapter 22

# CHANGES IN THE CHEMICAL COMPOSITION OF BLOOD

The effects of various physiological and pathological factors upon the composition of the blood have been discussed in several connections. They will now be collected, and other facts in the same category will be added for their systematic study. The investigation of "blood chemistry" has grown tremendously in the last quarter century and has proved a great aid to the physician and surgeon. The chief reason for this remarkable advance has been the development of analytical methods, most of them colorimetric, for the determination of the minute amounts of substance present in the small samples of blood ordinarily available. For example, creatinine is found in normal blood in a concentration of 1 or 2 mg. per 100 ml. of blood. The quantitative analysis of creatinine can easily be performed on 1 ml. of blood; i.e., an amount containing from 0.01 to 0.02 mg. of creatinine! These methods have been developed by a number of biochemists, beginning with Folin, and including, among many others, R. Benedict, van Slyke, Hagedorn and Jensen, Wu, Myers, Kramer and Tisell, Somogyi, Bloor, and Knudson. The information which these methods yield is particularly valuable in many conditions in which other laboratory procedures are ineffective. Cytology, serology, and bacteriology are of little help in diabetes, gout, nephritis, and acidosis. By the same token, the methods of blood chemistry are only useful to a limited degree in the differential diagnosis of infections, toxoplasms, or allergies.

## GENERAL COMPOSITION OF BLOOD

There is no need to repeat what was said in Chapter 8 concerning the relations of serum, plasma, and the formed elements. The following constituents are found in whole blood, some in the plasma, some in the formed elements, and some in both.

- Water
- Gases— $O_2$ ,  $CO_2$ , and  $N_2$
- Proteins—Serum albumin, serum globulins, fibrinogen, hemoglobin
- Lipids—Fat, cholesterol, cholesterol esters, lecithins
- Carbohydrates—Glucose, perhaps others
- Nitrogenous products—Urea, uric acid, creatine, creatinine, ammonium salts, amino acids
- Inorganic salts—
  - (Positive radicals) Na, K, Ca, Mg,  $NH_4$
  - (Negative radicals) Cl,  $CO_3$ ,  $HCO_3$ ,  $PO_4$ ,  $SO_4$
- Lactic acid
- Ketone bodies
- Enzymes
- Bile pigments, bile salts, etc.

The composition of normal blood, that is, the usual range of values found under standard conditions, is given in Table XLIX. "Standard conditions" means that the subject has been on an ordinary mixed diet and the blood sample is taken before breakfast (that is, from twelve to sixteen hours after the last meal), with no undue exertion or excitement.

**Problems of Analysis.**—The methods of drawing blood and its analysis do not come within the province of this volume. Venous blood is most commonly employed, but arterial blood has occasionally been used, and if micromethods are available, small amounts may be taken from the finger, ear lobe, or, in

TABLE XLIX  
COMPOSITION OF NORMAL HUMAN BLOOD (POSTABSORPTIVE STATE)\*

WHOLE BLOOD	GM. PER CENT	
Total solids	19-23	
Water	77-81	
Hemoglobin		
Adult males	15.8	
Adult females	13.8	
Children	12.0	
Total nitrogen	3.5	
	VOL. PER CENT	
Carbon dioxide content (venous)	50-60	
Carbon dioxide content (arterial)	45-55	
Oxygen capacity	16-24	
Oxygen content (venous)	10-18	
Oxygen content (arterial)	15-23	
	MG. PER 100 ML.	
Uric acid	2-3.5	
Creatinine	1-2	
Creatine	3-7 (?)	
Glucose	70-120	
	(depending on method used)	
Total fatty acids	300-400	
Cholesterol	150-190	
Total acetone bodies (as acetone)	1-5	
Iron	52	
Nonprotein nitrogen	25-35	
Urea	22-33	
Urea nitrogen	10-15	
Amino acid nitrogen	5-8	
Ammonia nitrogen	0.1-0.2	
Undetermined nitrogen	4-10	
Lecithin (as lipid phosphorus)	10-12	
Chloride (as sodium chloride)	450-500	
Lactic acid	5-20	
PLASMA	MG. PER 100 ML.	MEQ. PER LITER
Fibrinogen	200-400	
Inorganic phosphorus		
Adults	3-4.5	1.7-2.5
Children	4-6	2.2-3.3
Chloride (as sodium chloride)	580-630	
(as Na)	226-248	99-108
(as Cl)	352-382	99-108
Carbon dioxide capacity	55-75 volumes per cent	20-33
SERUM	PER CENT	
Total protein	6.5-8.5	
Albumin	3.6-5.4	
Globulin	1.5-3.4	

\*Revised from Kleiner, I. S., and Dotti, L. B.: Laboratory Instructions in Biochemistry, ed. 3, St. Louis, 1951, The C. V. Mosby Co.

TABLE XLIX—CONT'D

SERUM	UNITS PER 100 ML.	
Amylase, Somogyi	80-160	
Acid phosphatase, Gutman	0.5-2.0	
Acid phosphatase, King	1.4-4.5	
Alkaline phosphatase (Bodansky units)		
Adult	2-3.5	
Child	5-14	
Alkaline phosphatase (King units)		
Adult	5-10	
Child	15-20	
Icteric index	4-6	
	MG. PER 100 ML.	MEQ. PER LITER
Bilirubin, indirect	0.2-0.8	
Calcium	9-11	4.5-5.5
Cholesterol, total	150-300	
Cholesterol, ester	105-210	
Ester: total (ratio)	0.7	
Creatinine	1.0-1.8	
Iron†	0.028-0.210	
Sulfate, inorganic (as S)	0.9-1.1	0.6-1.1
Phosphate	3-4.5	1-2
Magnesium	1.0-3.0	0.9-2.5
Potassium	16-22	4.1-5.6
Sodium	310-333	135-145
Uric acid	3.0-5.0	
RED BLOOD CELLS		
Hemoglobin	35%	
	MG. PER 100 ML.	
Potassium	420	
Sodium	25	
Magnesium	6.6	
Calcium	Small amount	
VITAMINS		
Niacin	0.35-0.53 mg. per 100 ml. of blood	
Ascorbic acid	0.7-2.5 mg. per 100 ml. of blood	
Thiamine plus cocarboxylase	10 gamma per 100 ml. of blood	
Thiamine	1 gamma per 100 ml. of plasma	
MISCELLANEOUS CONSTITUENTS*		
	MG. PER 100 ML.	
Copper (plasma or serum)	0.086-0.161	
Fluorine (whole blood)	0.28	
Iodine (protein bound) (plasma or serum)	0.006-0.008	
Lead (whole blood)	0.009-0.05	
Manganese (whole blood)	0.005-0.02	
Silica (soluble) (whole blood)	1.5 (as SiO <sub>3</sub> )	
Silica (total) (whole blood)	9.0 (as SiO <sub>3</sub> )	
Zinc (plasma or serum)	0.12-0.48	

\*Krebs, H. A.: Chemical Composition of Blood Plasma and Serum, Ann. Rev. Biochem. 409, 1950.

case of babies, from the heel. Unless serum is desired, clotting must be prevented. This is accomplished by using a slight excess of sodium, potassium, lithium oxalate, sodium citrate, or sodium fluoride. In some instances the blood should be taken under oil to prevent the loss of gases. Analysis should be begun promptly because some of the constituents are altered on standing. In most determinations the proteins must first be removed. This is not the case, of course, when the proteins themselves are to be determined, nor for the estimation of the gases. A number of protein precipitants are useful in this connection, e.g., picric acid, zinc hydroxide, alcohol, but the one which is most versatile" is tungstic acid (sodium tungstate with sulfuric acid). This is the



precipitant employed in the Folin-Wu procedure, and the clear colorless filtrate may be used for a number of different determinations. Neuberg and associates have recently proposed the use of perchloric acid,  $\text{HClO}_4$ , as a protein precipitant because it alone possesses all of the following properties. It precipitates only proteins, it does not interfere with the analysis of any known constituent of plasma, it may be removed easily if present in excess, and it is not costly or dangerous to use.

In blood chemistry it is the concentration of the substance under investigation which is of interest. Therefore results are expressed in percentage (i.e., grams per 100 ml. of blood or serum); in milligrams per 100 ml. of blood; in the case of gases, as milliliters of the gas per 100 ml.; and in the case of trace constituents, in gamma per 100 ml.

## GLUCOSE

The carbohydrate of blood is generally accepted to be D-glucose. There may be traces of other sugars present, and there are undoubtedly other reducing substances present which react with the reagents most commonly employed. Several recent methods give results, which more nearly resemble the "true sugar" values. This can be obtained most accurately by determining the total reduction by any good method, then subtracting from this the amount of reduction which remains after a second sample has been subjected to fermentation by yeast. The older methods give results for normal blood varying from 90 to 120 mg. per 100 ml. (0.090 to 0.120 per cent); the newer methods give slightly lower results, namely, 70 to 100 mg. per 100 ml., which is probably nearer the true sugar value. These figures, of course, refer to the composition of the blood before breakfast, after a twelve- to sixteen-hour fast. Half an hour to two hours after a meal the glucose may rise to 120 mg. or even higher (see Fig. 50, Chapter 16). If the meal is particularly rich in carbohydrate, a value as high as 160 mg. may be found. The need for taking blood under fasting conditions is evident. Excitement, pain, and other emotional disturbances tend to raise the level, while starvation lowers it.

Pathologically, blood sugar values above normal, i.e., hyperglycemias, are more frequently encountered than those below normal, or hypoglycemias. In mild diabetes mellitus, the blood sugar is likely to range from 100 to 200 mg., and severe cases show values from, say, 250 mg. upward. Values of 300, 400, 500 mg. in untreated patients are not uncommon, and figures even in excess of 1,000 mg. have been observed. Usually at about a level of 160 mg. the sugar "spills" over into the urine; that is, the amount of sugar which passes through the glomerulus is too great for the absorbing mechanism of the tubules. However, sometimes the "renal threshold" is raised and values of 200 mg. or more are found in which no glucose is excreted in the urine. It is quite apparent that urinary sugar analyses cannot give as definite information regarding the metabolic condition of a diabetic patient as blood analysis. The physician today follows the result of his treatment of a diabetic patient by having the blood sugar determined at suitable intervals.

An increased activity of the islands of Langerhans is seen in hyperplasia of this tissue. Thus, in adenoma or carcinoma of islet tissue there occurs hyperinsulinism which causes marked hypoglycemia. Surgical removal of the tissue may result in cure.

The pancreas also contains a hyperglycemic factor, called by deDuve, IGF, and recently designated "glucagon." (See page 435.) This is found in most insulin preparations. It causes a transitory rise in blood sugar if the insulin is injected intravenously and if there is an adequate store of liver glycogen. It has no hyperglycemic effect after subcutaneous injection.

In renal diabetes or renal glycosuria, a glycosuria occurs with no deviation of the blood sugar from normal. This is a relatively uncommon condition. The defect is not one of metabolism, since the blood sugar never rises appreciably. Apparently either the glomerulus permits glucose to pass more freely or the tubules do not absorb it at a normal rate. Whatever the reason, the threshold is lowered. Some of the criteria, upon which a diagnosis may be based, differentiating renal diabetes from diabetes mellitus, are: (1) fasting blood sugar within normal limits, (2) a normal or below normal glucose tolerance curve, (3) glucose present in urine voided before and after meals, and (4) no effect upon glycosuria by injection of insulin.

The blood sugar level is changed in certain other conditions. In nephritis the blood sugar is often raised to perhaps 160 to 170 mg., and is rarely over 200 mg. However, a hyperglycemia of this sort is not easily mistaken for diabetes mellitus. The clinical picture is different and the urine is usually free from glucose, even after a glucose tolerance test is made. Such a test will often aid in diagnosis. (See page 437.)

Since some of the effects of pituitary secretion are in opposition to the effect of the pancreas, we find hyperglycemia in acromegaly (hyperpituitarism), and hypoglycemia in later stages of the same condition, when there is a diminished gland function. Neither is very pronounced.

Hyperthyroidism results in hyperglycemia in a small percentage of all cases. It is said to be due to increased hepatic glycogenolysis. Since the basal metabolic rate is high in such conditions, the increased metabolic activity usually takes advantage of the abundance of glucose. It is only when glycogen is in great excess in the liver that the blood sugar can rise much above the normal levels in hyperthyroidism. Many hyperthyroid patients show some glycosuria, however, whatever the level of glycemia. Surgical removal of thyroid tissue usually brings the carbohydrate metabolism back to normal. Hypothyroidism is frequently accompanied by hypoglycemia, and administration of thyroid gland preparations raises the level to normal again.

Hyperglycemia may occur in increased intracranial pressure, as a result, for example, of skull fracture, cerebral hemorrhage, or brain tumor. This, perhaps, may have an origin similar to that of Claude Bernard's piqûre experiment. Probably through nervous stimulation adrenaline is released and glycogenolysis stimulated.

The "general adaptation," or stress, syndrome is responsible for many hyperglycemic reactions. (See page 637.) As a result of pain, fear, cold, in-

fection, or other stresses, there is an increased secretion of the adrenocorticotrophic hormone. This, in turn, stimulates the adrenal cortex to put out more "glucocorticoids," the steroids which have a blood sugar-raising effect. For the increased metabolism which takes place in fever, a supply of fuel is required, hence the increase in blood glucose, which occurs in infections, usually accompanied by fevers, is a fortunate circumstance.

The hyperglycemias which occur in ether and chloroform anesthesia are due to increased epinephrine secretion, leading to heightened glycogenolysis. Morphine has a similar effect, and probably the hyperglycemia of asphyxia is explained on the same basis.

Hypoglycemias are frequently encountered in conditions in which the liver is considerably involved. Such a state may result from phosphorus, chloroform, or carbon tetrachloride poisoning. Acute yellow atrophy of the liver, acute diffuse necrosis of the liver, and carcinoma of the liver similarly cause very low blood sugar values, because of the extensive destruction or disabling of the liver cells, with a consequent deleterious effect upon glycogenesis and glycogenolysis. In severe burns a marked hypoglycemia may occur. Toxic products are known to circulate as a result of burns, but just how these affect carbohydrate metabolism is not understood. Hypoglycemia may be found in newborn infants of diabetic mothers, even to the extent of causing convulsions. The explanation is interesting. It is suggested that the pancreas of the fetus, while in utero, has been stimulated to furnish the mother with insulin in addition to providing for its own needs. It continues this hypersecretion after birth, and before adjustment to its own normal state can take place, the excess of insulin produces the dangerous fall in glycemia.

### NONPROTEIN NITROGENOUS CONSTITUENTS

Except for the amino acids, the nonprotein nitrogenous constituents are waste products, and these are of great importance from a clinical standpoint. The amounts present normally are as follows:

	MG. PER 100 ML. BLOOD
Total nonprotein nitrogen	25-35
Urea nitrogen	10-15
Creatinine	1- 2
Uric acid	2- 3.5
Creatine	3- 7(?)
Amino acid nitrogen	5- 8
Ammonia nitrogen	0.1- 0.2
Undetermined nitrogen	4-10

#### Total Nonprotein Nitrogen

The total nonprotein nitrogen is sometimes determined in order to get some idea of the retention of nitrogenous products generally. However, it is apparent that a small deviation, such as 2 mg., from the normal 25 to 35 mg. would not be at all significant. But these small deviations might be due to real changes in uric acid or creatinine which would be important if they were known. Since the procedure for the determination of the total nonprotein nitrogen (N.P.N.) is not as simple as that for urea, for example (which has



out the same significance), the former determination is not being called for very often at present. Urea, uric acid, and creatinine are the constituents of greatest moment and have been extensively studied.

### Blood Urea

Blood urea is usually expressed as "urea nitrogen." The normal range is from 10 to 15 mg. urea N per 100 ml. of blood. Some authorities and an even narrower range, namely, 12 to 15 mg., and recently it has been shown that there is a correlation between age and urea concentration, the urea increasing from middle age to old age. An increase in the concentration above normal is due to one of the following factors: (1) failure of the body to eliminate urea; (2) excessive urea formation (i.e., increased protein metabolism); and (3) concentration of the blood (i.e., dehydration). These three mechanisms are often interlinked; that is, more than one may be operating at the same time.

In nephritis, that is, glomerulonephritis, there is a net diminution in the filtration of urea. Less urea is eliminated and blood urea consequently rises. This usually does not occur unless the kidney lesion is rather severe. A urea N of 20 or 25 mg. should be viewed with suspicion, but since the level of urea fluctuates more than that of uric acid or creatinine, it may not be an unequivocal indication of renal dysfunction. In the terminal stages of chronic nephritis or in severe acute attacks, values as high as 200 mg. may be found.

Urea elimination is, of course, diminished whenever urine formation is interfered with. This may be caused by physiological hindrance to flow, as occurs in obstruction of the ureters; in bichloride of mercury poisoning, which blocks renal secretion; and in heart and circulatory disturbances, with their effects upon the blood supply to the kidney. In the same category would come any condition in which there is actual destruction or loss of renal tissue. The blood urea will rise in all such cases, but if the normal state can again be restored, even in part, it will be reflected in a lowering of the blood urea level.

In many cases it is important to have some means of evaluating the ability of the kidney to remove waste products from the blood. Several "kidney function" tests have been devised for this purpose, and many of them utilize blood urea determinations as part of the routine. The urea clearance test and the ratio of blood urea to blood N.P.N. are among these and are described in Chapter 25.

Intensified protein metabolism, with normal or subnormal urea elimination, will result in a higher blood urea. Usually increases in blood urea originating in this way are not as great as from nonelimination. Extremely high protein intake might result in such an increase, which would soon fall to a lower level after resumption of a normal dietary. Similarly, the increased catabolism of body protein may have the same effect. Toxic and febrile conditions which cause breakdown of tissue protein are often accompanied by high blood urea concentrations. The high blood urea which follows a severe hemorrhage, or continued hemorrhage into the stomach or duodenum is partly explained on the same basis, although other factors may play some part, such as impairment of renal function and dehydration.

Dehydration of the blood, caused by loss of body fluids, is accompanied by an increase in blood urea. This is only partly a result of the concentration per se; that is, the fact that the normal amount of urea is dissolved in a smaller amount of fluid. Perhaps more important is the impairment of renal circulation which goes hand in hand with a more concentrated blood. Examples of conditions which result in dehydration are excessive sweating, vomiting, and diarrhea. This is also probably the major reason for the high blood urea which follows intestinal obstruction and which is the most constant blood change noted in that condition. Obstruction may be present for some time before the blood urea rises, i.e., the increase in blood urea is secondary to the obstruction. A marked loss of fluid into the gastrointestinal tract, usually with persistent vomiting, always accompanies intestinal obstruction. As a result, dehydration occurs with elevation of the blood urea. Similarly, in surgical shock, in Addison's disease, and in all other syndromes involving blood concentration there is seen an increase in urea.

The blood urea is seldom found to be much below normal. Values as low as 5 to 10 mg. per 100 ml., however, may occur when there is extensive liver damage, since the liver is the site of urea formation. This is more likely to be the case in acute conditions, such as acute yellow atrophy of the liver and the hepatic poisonings, than in chronic states, because in the latter there is usually some normal hepatic tissue to carry on for the rest of the organ. In diuresis, the large urine volume carries urea with it and, since this substance is not actively reabsorbed by the tubules, the blood urea is bound to fall. In uncontrolled diabetes mellitus the diuresis results in subnormal values, but in diabetic coma the acidosis has an opposite effect because of the inhibiting action of the acetone bodies upon the kidneys. Another condition with slightly low blood urea values is lipid nephrosis.

In normal pregnancy the urea nitrogen seems to fall slowly until about the eighth month, after which it rises slightly. During the last three or four months, values as low as 6 mg. may be encountered. The cause of this is not understood. It may indicate that the amino acids are being utilized by the fetus instead of being deaminized and converted into urea by the liver.

### URIC ACID

The normal uric acid range is usually stated to be about 2 to 3.5 mg. per cent for whole blood. However, this is not all uric acid; and these figures are about one-third too high. It is much more accurate to use serum for uric acid determinations because the cells contain more of the substances which react like uric acid to give the high values. Moreover, most of the uric acid is in the serum and most of the fluctuations occur there. Serum uric acid normally varies from 3.0 to 5.0 mg. per 100 ml., with an average normal value of about 4 mg. per cent.

Although uric acid is the end product of nucleic acid, and, in particular, of purine metabolism, the ingestion of large amounts of purine-yielding foods has very little effect upon the blood uric acid normally. Moreover, under normal conditions no other factors seem to affect this level appreciably. Violent muscu-



Exercise raises it slightly, at most 1 mg. In normal pregnancy there is no change until the onset of labor, when there is a temporary increase of 1 or 2 mg. The cause is not known. In starvation the blood uric acid may be considerably increased up to as much as 10 mg. This is believed to be due to the accelerated destruction of tissue cells, which occurs in starvation, and to an inhibition of uric acid excretion by the kidneys. The latter probably results from the acidosis, which always accompanies starvation.

Abnormally, the blood uric acid may be increased for one, or more, of three reasons. There may be (1) a diminished excretion of uric acid, (2) increased production of uric acid, or (3) diminished destruction of uric acid. Folin showed that when uric acid was administered intravenously, an average of 50 per cent was destroyed.

Diminished excretion of uric acid is seen in nephritis. According to Myers, Lane, and Lough, there is a definite order in which uric acid, urea, and creatinine are "retained" by the kidney. They showed that uric acid is the most difficult to excrete and is the first to rise when the kidneys are damaged; next, urea is retained; and, finally, the substance which is most easily eliminated, creatinine. An elevation of blood uric acid is therefore one of the earliest signs of kidney disease. Although all observers do not agree that this is invariably the case, it appears to hold rather generally. The ingestion of purine-rich foods raises the blood uric acid in nephritis, although in normal individuals it has little or no effect. Besides nephritis, any other condition which would lead to obstruction of urinary flow or suppression of urinary secretion would result in a retention of uric acid. This is just as true in the case of uric acid and creatinine as it is for urea. Consequently, we find high uric acid in the blood in prostatic diseases, ureteral calculus, and cardiac decompensation.

In leukemia there is an increased formation of uric acid because of the augmented nuclear metabolism resulting from the formation and destruction of leucocytes. The blood uric acid rises as a result of this overproduction. In polycythemia and lobar pneumonia the same thing happens. During remissions of pernicious anemia there is an increase in this constituent, due probably to the metabolism of the nuclear material lost by the new red blood cells. Besides the formation of uric acid as a result of the metabolism of preformed purines, there may be an increased production of uric acid by the synthetic route. This will be discussed below.

Since the site of uric acid destruction in the body is probably the liver, one would expect to find blood uric acid levels to be high in liver disease. This is not the case in general. In very extensive liver damage high figures have been found, but generally there appears to be enough functioning liver tissue to destroy the usual amount of uric acid. It was formerly thought that gout was due to a defect in uric acid destruction, but this is probably not the case. Instead, it is maintained that there is a slightly increased uric acid destruction, perhaps because of the somewhat higher level of uric acid in the blood. Gout is characterized by acute attacks of painful and swollen joints lasting several days, followed by longer periods of remission. Careful analyses of serum, rather than whole blood, have convinced Talbott, Jacobson, and their colleagues that the uric



acid levels in individuals who have gout are appreciably higher than in those who do not have it. This is contrary to former views. For example, in 100 persons who did not have gout, the serum uric acid ranged from 1.9 to 6.7 mg. per 100 ml., while in 21 who had gout the range was from 5.2 to 14.8 mg. They interpret this as indicating an increased formation of uric acid rather than a decreased excretion or destruction. Adlersberg found similar differences, and stresses the fact that a part of the uric acid is bound, probably to protein. In gout and in hepatic damage, not only is the total uric acid higher than normal, but the proportion of bound uric acid is also higher. It is suggested that the abnormal "fixation" of uric acid in blood and tissues might result in a diminished elimination of this substance by the kidney, with a resulting hyperuricemia.

Stetten showed that there was a considerable difference between the "miscible pool" of uric acid under normal conditions and under gouty conditions. The miscible pool of uric acid is defined as that quantity of uric acid in the body which is capable of mixing promptly with intravenously injected, tagged uric acid. It is determined by estimating the proportion of tagged uric acid in the serum and in the urine after such an injection. Normal subjects had an average pool of 1,131 mg., while patients suffering from gout had from 4,700 to 31,000 mg., depending upon the severity of the disease, the diet, and medication. These large quantities, found in gout, are far greater than could possibly be presumed to be in solution in body water. It is therefore concluded that some of the uric acid must be in the solid phase, probably in the superficial layers of the urate masses (tophi), situated in the cartilages. This indicates that in gout there is a continual solution and precipitation of urate at the interface, between the tophus and the body fluid. The cause of this increase appears to be a greater synthesis of uric acid by the body, as suggested above. Additional evidence for this view was obtained when isotopic ( $N^{15}$ ) glycine was fed to normal persons, and about 0.15 per cent was recovered in the urine as isotopic uric acid. Under the same conditions, gouty persons excreted 0.5 per cent.

Just what causes the pain in an acute attack of gout is undecided. Uric acid may crystallize out in tissues without causing pain or inflammation. Magnus-Levy has suggested that it is not the deposition of uric acid which is painful but the resolution of such deposits. It should be noted that in uncomplicated gout the other nonprotein nitrogenous constituents are not increased in amount.

In eclampsia the uric acid level is considerably elevated. The cause of this is not understood.

A decrease in the uric acid level of the blood almost never occurs.

## CREATININE

The normal level of creatinine is quite constant for a given individual and is not affected by age, diet, or other physiological factors. The normal range is from 1 to 2 mg. per 100 ml. blood with a larger proportion of values nearer 1 than 2 mg. Creatinine is so easily eliminated that any figure above 2 mg. may be considered abnormal. The creatinine content of serum is slightly lower than that for whole blood, because of the presence in the red blood cell of noncreatinine material which gives the same reaction as creatinine. Therefore, serum is used

more frequently than whole blood, but the interpretation of the results is exactly the same. The creatinine range in serum is from 1.0 to 1.8 mg. per 100 ml.

Creatinine may be increased in most of the circumstances in which urea is increased. Since the production of creatinine involves quite a different mechanism from that of urea, although in a sense, it is a phase of protein metabolism, its level in the blood is not as closely related to protein metabolism as the urea level is. Factors which modify the excretion of urea by the kidney have a similar effect upon creatinine. Quantitatively, however, there are great differences. In chronic nephritis, creatinine is the last of the three important nonprotein nitrogenous substances to rise. A value above 3 mg. is usually accompanied by retention of uric acid and urea. The prognosis is not bad, however, until the level of 4 mg. is reached. Above this point there is little chance for improvement in the patient, and above 5 mg. a fatal outcome within a year is to be expected. This prognostic scale does not hold for *acute* nephritis. Here high creatinine values may be reached for short periods, with a return to lower values when the acute attack has subsided.

High creatinine values, without prognostic significance, occur in conditions of urinary obstruction, cardiac difficulties, and intestinal obstruction. These have been discussed above in relation to blood urea, and the same physiological and pathological factors prevail in the case of creatinine. These facts indicate that creatinine in itself has nothing to do with causing the condition in chronic nephritis. It is merely an index of the gravity of the situation.

**Amino Acids.**—The amino acid of blood is not ordinarily determined clinically. The normal range is from 5 to 8 mg. per 100 ml. Its estimation, however, may be of value in the diagnosis of acute yellow atrophy of the liver, or of any condition in which there is tremendous damage to the liver. Since the liver is the location of the deamination of the amino acids and production of urea, it is logical to expect that in the absence of functioning liver tissue the N.P.N. will be made up of more amino acids and less urea. This is the case if *most* of the liver tissue is destroyed. Besides acute yellow atrophy, such destruction may result from phosphorus, chloroform, carbon tetrachloride, cinchophen, and arsphenamine poisoning. Elevation of the amino acid level may also occur in severe renal dysfunction and in burns. Lowered levels are seen in certain infectious diseases, nephrotic crises, and in malnutrition.

## CHOLESTEROL

Although the function and intermediary metabolism of cholesterol are not well understood, a few of the known facts may be reviewed briefly. The animal sterols are probably absorbed from the intestinal tract along with the neutral fats and other lipids. They form esters with the fatty acids and thus act as a vehicle for their carriage in the blood stream. Some cholesterol is recovered in the disintegration of red blood cells, but more is synthesized by the body. Cholesterol synthesis occurs in the liver, in the skin, and in other tissues and appears to be inversely proportional to the amount of cholesterol available in the food. It is synthesized from acetate, or any substance which can yield acetate. This has been shown by isotope experiments of various types. (See also page 466.) Besides being a carrier of fatty acids, cholesterol has a number of other important physiological effects. It is an antihemolytic agent, counteracting the hemolytic action of bacterial toxins, snake venoms, bile salts,



and other hemolysins. It is a precursor of dehydrocholesterol, which is a precursor of vitamin D, of cholic acid, which forms a part of the bile acids, and, perhaps, of all of the steroid hormones. It is a primary constituent of all cells and, in some cases, of extracellular material. Because of its unusual physical properties, it undoubtedly modifies the permeability of the cell membrane and insulates nerve structures during the passage of electrical nerve impulses. The pathways of excretion appear to be by way of the bile, the intestinal mucosa, and, to a very small extent, by the urogenital mucosa into the urine.

The cholesterol is more or less evenly divided between the plasma and the erythrocytes. However, in the blood cells, and, indeed, in the tissue cells, the cholesterol is almost all free, while in serum or plasma about 70 per cent is present as cholesterol esters of fatty acids. Serum or plasma studies are of more clinical value than those of whole blood. From 150 to 250 mg. per 100 ml. is perhaps the range for total cholesterol in normal serum. Each laboratory will have its own normal figures for the particular method in use. Some of the older but still very widely used methods yield a comparatively low range, namely from 140 to 200 mg. The studies of Gofman indicate that some of the cholesterol is present as a constituent of large aggregates, or macromolecules, of lipoproteins. (See page 467.)

The serum cholesterol is remarkably constant normally. There is no appreciable change after the ingestion of large quantities of lipids, and the effect of food generally is of little moment. The statement frequently made that blood cholesterol rises markedly after feeding large quantities of lipids is based upon experiments upon dogs and rabbits, in which lipid metabolism appears to be quite different from that in man.

Total cholesterol values vary somewhat with age. The minimum values are found in late adolescence, increasing until about age 60, then decreasing in old age. (Keyes.) In pregnancy the serum cholesterol rises, but no good reason for this can be given. The menstrual cycle is also accompanied by changes in blood cholesterol. At or near menstruation there is a fall in the level, with a hypercholesterolemia either just before or just after it.

Hypercholesterolemia commonly accompanies hyperglycemia in diabetes mellitus. Along with the rise of cholesterol there is also an increase in all the lipid constituents. Although the cholesterol does not always parallel the glucose of the blood, it is claimed to be a better index of the severity of the disease. Values of 300 mg. or over are frequent, and levels of 500 mg. or even higher occur in patients with severe untreated diabetes with ketosis. The administration of insulin does not lower the blood cholesterol immediately as it does the blood sugar. The effect requires a period of days or weeks and is therefore probably an indirect one (Bruger and Mosenthal).

In "lipoid nephrosis" the blood cholesterol is regularly increased. This is a type of kidney condition in which the tubules are edematous and show degenerative changes with lipid deposits in the cells. It is, however, now considered to be a type of glomerulonephritis, in which protein escapes through the glomerulus. In spite of the abnormal appearance of the tubular cells, they may function almost normally. Among the symptoms are



oliguria, hypoproteinemia, proteinuria, and edema. The cholesterol of the blood rises to very great heights. Figures of from 500 to 800 mg. are not uncommon. The other lipids also increase but not to the same degree as cholesterol. The proportion of cholesterol esters, which normally is about 70 per cent of the total, rises to from 80 to 90 per cent in lipoid nephrosis.

In jaundice due to obstruction of the bile duct, the blood cholesterol rises and seems to parallel the amount of bilirubin in the blood. This is biliary cholesterol, prevented from entering the intestine by the obstruction.

Plasma cholesterol bears an inverse relationship to thyroid activity, hypothyroidism being associated with the high values, and hyperthyroidism with the low ones. The appropriate treatment tends to bring the level to normal. Thus, blood cholesterol determinations may be of value from a diagnostic standpoint and as an aid in following methods of treatment.

In pernicious anemia, as well as in some other types of anemia, there occurs marked hypocholesterolemia. The cholesterol level may be as low as 50 mg. per 100 ml. The degree of hypocholesterolemia bears no relation to the severity of the disease, but the concentration of cholesterol is usually low if the hemoglobin is below 8 Gm. per 100 ml. Anemia resulting from hemorrhage and severe aplastic anemia (that is, anemia resulting from lack of formation of cellular elements in the bone marrow) are, on the other hand, accompanied by high cholesterol levels.

In hemolytic jaundice there is a low blood cholesterol in contrast to the high values in obstructive jaundice, and in acute liver diseases, such as infectious hepatitis and acute yellow atrophy, there are also abnormally low cholesterol values and particularly a diminished proportion of cholesterol esters. Low values are also seen in infections and in malnutrition.

## PROTEINS

### Hemoglobin

The function of hemoglobin does not need to be described again, and its great importance must be quite apparent. The normal values vary with age and sex. In adult males the average is about 15.8 Gm. per 100 ml. and in females it is about 2 Gm. lower. In the newborn infant it is very high, from 18 to 23 Gm. per 100 ml., but falls rapidly to a minimum figure of about 13 Gm. at the sixth month. It remains at a low level until the end of the second year and slowly increases until it reaches the adult figure. In extreme old age there is a slight decrease to about 14 Gm. per 100 ml.

The correct method of reporting hemoglobin values is in the manner just given; i.e., in grams per 100 ml. of blood. The usual clinical custom, however, has been to report the hemoglobin in relation to the assumed normal. That is, a value of "100 per cent" meant a normal value. The different instruments, however, did not agree on the equivalent of this 100 per cent in grams of hemoglobin per 100 ml. of blood. For example, in the Dare hemoglobinometer 13.77 Gm. of hemoglobin per 100 ml. of blood was used as the standard, while the Sahli took 17.2 Gm. per 100 ml. It was thus possible to have a hemoglobin value

of 120 per cent or 96 per cent for the same sample of blood (with a content of 16.5 Gm. of hemoglobin per 100 ml.), depending upon which method was employed. The normal average figures given above (15.8 Gm. per cent for men and 13.8 Gm. per cent for women) are the mean of observations made throughout the world (Myers and Eddy). For greatest accuracy hemoglobin should be determined by the Van Slyke and Neill method which estimates the oxygen capacity. For routine work this is too time consuming and a colorimetric method, or the copper sulfate specific gravity method, is usually employed.

Hemoglobin values are influenced by other physiological factors besides age and sex. There is a diurnal variation in normal persons amounting to as much as 20 or 30 per cent of the average concentration. Exercise causes an increase in the hemoglobin, because of splenic contractions which force more red cells into the circulation; if long continued, however, the hemoglobin may be destroyed to some extent and consequently the level may fall below normal. Dehydration, from sweating or diarrhea, raises the hemoglobin, while an increase in the water content of the body decreases it. Cold baths and emotional excitement are also causes for rise in hemoglobin. Consequently, if an accurate picture of the hemoglobin of a person is desired, all of these facts should be borne in mind. That is, the blood sample should be taken at a standard time and under standard conditions—say before breakfast, without a morning bath, and with the avoidance of mental or physical stress. In pregnancy the needs of the fetus cause a deficit in the hemoglobin of the mother. A return to normal occurs after parturition, if the food contains the requisites for building hemoglobin.

Pathologically, decreases in the hemoglobin content of blood are seen in anemias, hemolytic jaundice, and after hemorrhages. The diminished total circulating hemoglobin is due either to a diminution in the number of cells containing a sufficient content of hemoglobin in each cell, to a diminished hemoglobin content of the red cells but no change in their numbers, or to a combination of these changes. It is therefore essential to know, not only the hemoglobin, but also the red cell count. The two have been correlated in the "color index" of blood.

$$\text{Color index} = \frac{\text{Hemoglobin in grams per 100 ml.}}{\text{Red cells in millions per cubic millimeter}} \times 0.31$$

The factor 0.31 is derived from the ratio of the approximate normal value of 5 millions of red cells to the approximate normal value of 16 Gm. of hemoglobin per 100 ml. Thus the normal color index for these typical normal values would be 1, and values much below this indicate a subnormal content of hemoglobin in the cells. "Hypochromic" anemias have low color indices and "hyperchromic" ones have high indices.

In nutritional anemias, chlorosis, and toxemias of pregnancy there are low color indices. In pernicious anemia, on the other hand, the color index is high, with large and irregularly shaped red cells. The low total hemoglobin is therefore due to a smaller number of these large red cells. In hemolytic jaundice there is a hypochromic condition due to both a low count and a low hemoglobin

concentration of the cell. Anemia following hemorrhage results in a loss of numbers of cells, of course, at first, and the new cells formed are likely to possess a low content of hemoglobin, and thus there is a low color index.

Elevation of the hemoglobin concentration occurs whenever the red cells are greatly increased in numbers. These conditions are termed "polycythemias." The color index, however, may be very low, as is the case in idiopathic polycythemia. The cell count increases tremendously and, although the total hemoglobin also increases, it does not do so to the same extent. In diarrhea and whenever dehydration is marked, the concentration of blood due to water loss brings about a rise in hemoglobin values.

It may also be mentioned at this point that either methemoglobin or sulfhemoglobin may be found in the blood of patients who have been treated with sulfanilamide. The drug does not act upon hemoglobin in vitro, and therefore it is assumed that it acts upon some tissue in such a way as to cause the production of a substance which effects the transformation in question.

### Albumins, Globulins, and Fibrinogen

The total blood plasma proteins have a normal range of from 6.3 to 8.0 Gm. per 100 ml. In this is included fibrinogen, albumin, and globulins. The fibrinogen is normally from 0.2 to 0.4 Gm.; the albumin, from 3.9 to 5.3 Gm.; and the globulins, from 1.3 to 3.4 Gm. The globulins, as ordinarily determined, do not include fibrinogen. These values are for determinations made by chemical methods. For example, the albumin, globulin, and fibrinogen fractions may be separated from each other by salting out with different concentrations of sodium sulfate. The protein in each fraction is then estimated colorimetrically or by the Kjeldahl method. There is, however, a growing tendency to separate various fractions by electrophoresis (see page 171), since the chemical method may be tearing apart protein complexes, which it is believed are preserved in electrophoresis. Table XXIII compares the protein

TABLE L

AVERAGE NORMAL PLASMA PROTEIN CONCENTRATION VALUES FROM BIRTH TO MATURITY\*

AGE†	PLASMA PROTEIN‡			
	TOTAL PROTEIN GM./100 ML.	ALBUMIN§ GM./100 ML.	GLOBULIN GM./100 ML.	FIBRINOGEN GM./100 ML.
Premature infants	4.55	3.55	1.01	0.27
Full term infants	5.11-5.70	3.76-3.79	1.34-1.66	0.24
Birth to 1 year	6.10	4.97	1.38	0.28
1 to 4 years	6.94	4.59-4.83	2.03	0.21
5 to 12 years	7.30	5.0	2.4	0.28
Under 15 years	7.16	4.72	2.49	Included with globulin 0.21
Adults	6.94-7.18	4.59-4.70	2.03-2.54	
Adult range (95%)	6.3-8.0	3.9-5.3	1.3-3.4	Included with globulin

\*Adapted from Metcoff, J., and Stare, F. J.: New England J. Med. 236: 26, Jan. 2, 1947.

†No significant variation with sex.

‡Values obtained are somewhat variable, depending on technic of determination. Most of the reported total protein values are derived from micro-Kjeldahl analyses, and the albumin and globulin from sodium sulfate fractionation.

§Albumin may vary somewhat with the season. The variation may be a manifestation of seasonal blood volume changes.



composition of plasma when analyzed by the two methods. It is quite evident that the two sets of figures do not agree, and these differences may prove to be of fundamental importance.

The plasma proteins are low in early infancy, particularly the globulin fraction, but at about the age of 18 months they reach the concentration seen in adult life. (See Table L.) In pregnancy there is a decrease in the *concentration* of total protein, but, because of the greater blood volume, the *absolute amount* of protein is, in fact, increased. (Novak and Lustig.) During the first six months of pregnancy the albumin fraction gradually diminishes and then rises to normal at term. When the albumin value is low, the globulin and fibrinogen are elevated. The menstrual period is accompanied by an increase in fibrinogen. Exercise causes a rise in plasma proteins as a result of (1) the increased number of red cells and consequent diminution in fluid volume and (2) the sweating which brings about dehydration.

On page 169 the colloidal osmotic effect of plasma proteins was discussed, and it was shown why the albumins had a greater influence than the globulins in maintaining this pressure. Normally the serum albumin constitutes about 52-55 per cent of the total plasma protein, and about 80 per cent of the osmotic pressure is attributable to it. It is therefore evident that the albumin fraction is more important from this standpoint than the globulin. Until recently it was felt that the ratio of albumin to globulin (the A:G ratio) was clinically significant and that a reversal of it was a pathological sign. That is, if the globulin became greater than the albumin in amount, the osmotic effect would be much less. This would be true if the total protein remained virtually unchanged, but it can easily be seen that a change in total protein concentration might compensate for any change in the ratio. Consequently the A:G ratio cannot in itself be considered a very informative criterion.

The serum albumin, because it is the smaller molecule, is more easily lost from the blood by leakage. It may go into the extravascular spaces as a result of trauma or shock or both. It may be excreted into the urine or lost from the surfaces of burns or wounds. Hemorrhage, of course, results in a loss of all blood proteins. Since the chief, if not the only, site of plasma protein synthesis is the liver, it is not surprising that a low serum protein is found whenever there is extensive liver damage, as in hepatitis and cirrhosis of the liver. Any disease which causes a depressed liver function will have a similar effect for a similar reason. In nephrosis the proteinuria is reflected in a lowered total serum protein content, but more particularly in a diminution of the albumin fraction, because the smaller albumin molecule more readily passes the renal epithelium. The edema which is likely to accompany nephrosis, and some of the other pathological states in which hypoproteinemia occurs, is due to the resulting fall in colloidal osmotic pressure of the blood. Many types of malnutrition lead to low plasma protein figures. Inadequate dietary protein is in this category. So, also, is an increased utilization of protein whenever a sufficient supply of calories is not available. Blood proteins are then utilized for energy. This may occur when intravenous infusions are given without supplying enough carbohydrate or fat. It may also ensue as a result

the increased metabolism in pregnancy, in lactation, and in the rapid growth of children. Decreased absorption of protein digestive products may also lead to decreased building of blood proteins. This may occur as a result of intestinal disorders.

Dehydration, whether as a result of insufficient intake of fluid or of loss of fluid, results in a more concentrated blood, with a higher content of proteins. Examples are intestinal obstruction, diarrhea, vomiting, Addison's disease, and severe diabetic acidosis. Diseases of the reticulo-endothelial system also give rise to a high content of plasma proteins, particularly the globulin fraction.

The fibrinogen fraction is increased in pneumonia and other infections. It also rises in cases of slight injury to the liver. This is probably because the damaged liver tissue is stimulated to greater activity in the production of this protein. On the other hand, if the hepatic lesion is extensive, the diminished liver activity results in a lowered fibrinogen content. Multiple myeloma, as will be remembered, is often characterized by the presence of Bence-Jones protein in the urine. This is a peculiar globulin and contributes to an increase in the globulin fraction of the plasma in this condition. The total protein is usually very high in this disease.

## CALCIUM AND PHOSPHORUS

Calcium and phosphorus are usually considered together, since disturbances of one usually result in disturbances of the other. As a rule they bear a reciprocal relationship to each other, a rise in one resulting in a fall of the other. This is not the case invariably, however. The normal content of calcium in serum ranges from 9 to 11 or 12 mg. per 100 ml. or 3 to 6 meq. per liter. (There is little or no calcium in the red cells.) About 50 or 60 per cent of this is diffusible; the remainder is probably linked to protein in a nondiffusible form. Most of the diffusible calcium is ionizable, and this is believed to be the physiologically active fraction. The proportions of the different fractions present are the resultant of an equilibrium between the total protein and the total calcium. Changes in the plasma protein may therefore be expected to have an influence on the concentration of diffusible calcium present. The phosphate also is composed of two fractions, an organic and an inorganic one. However, the organic phosphates are substances like phospholipids and do not have as much relationship to the inorganic phosphate as the bound calcium does to the diffusible calcium. The normal values for serum phosphate are from 3 to 4.5 mg. per 100 ml. for adults and from 4 to 6 mg. for children. This corresponds to about 1 to 2 meq. per liter. The physiological utilization of carbohydrate causes a slight decrease in serum phosphate because of the necessity of phosphorylation for this process. For this reason injections of insulin cause a slight temporary fall in phosphorus.

During bone formation a high phosphate is usually seen and sometimes a high calcium. This explains the higher phosphate figures for children and those found when fractures are healing.

The influence of the parathyroid gland and of vitamin D on calcium metabolism has been discussed in other chapters; therefore, no explanation will be given here. A lack of parathyroid hormone causes a fall in serum calcium and if parathyroid preparations are administered, there is an increase in the *diffusible* serum calcium. The phosphorus usually changes in the opposite direction. Vitamin D, or ultraviolet irradiation, raises the blood calcium but also raises the blood phosphorus, especially if it has been at a low level before treatment. In rickets the phosphate of the serum is usually low (down to 2 mg.) with a normal calcium value. In some cases the reverse is true and in still others both factors may be low. Whenever the product of the concentrations of calcium and phosphate, expressed as milligrams per 100 ml., falls below 30, rickets develops, but if it is over 40, rickets is absent or the healing process is taking place.

The greatest increase in serum calcium is seen in cases of tumor of the parathyroid gland. There is also a significant elevation in multiple myeloma. In the latter case this is due to an increase of the nondiffusible fraction and seems to be related to the excess of protein which is found in that ailment. Similarly the drop in nondiffusible calcium seen in nephrosis may be correlated with the hypoproteinemia.

### SODIUM AND POTASSIUM

The determination of sodium and potassium in blood plasma by chemical methods is difficult and time consuming. Thanks to the introduction and popularization of the flame photometer, the analyses of biological fluids for these elements is now an extremely rapid and accurate procedure. The method consists of atomizing a dilute solution of plasma into the flame of a Bunsen burner. Ignition causes light of characteristic wave length to be emitted, and the intensity of this light is measured photoelectrically. The range of normal values for human plasma is: sodium, 135 to 145 meq. per liter; potassium, 4.1 to 5.6 meq. per liter. Changes in plasma sodium serve to indicate, among other things, whether the plasma is isotonic, hypertonic, or hypotonic. Decreased plasma potassium levels may occur in a variety of conditions associated with potassium deficiencies, such as infantile diarrhea, alkalosis caused by pyloric stenosis, and the overzealous use of fluids which are not isotonic, or properly balanced. Increased potassium plasma levels may be found, less frequently, in certain clinical conditions such as prolonged anuria. Inasmuch as most of the body potassium is in the cells which take up and release potassium under various circumstances, alterations in plasma potassium levels are more difficult to interpret than plasma sodium levels. The progress of therapeutic measures and the effects of certain hormones can be assisted by such determinations and changes instituted as required. Some of these relationships were discussed in Chapter 18.

### CHLORIDE

Whole blood normally has a chloride content of from 450 to 500 mg. per 100 ml. as sodium chloride, but the determination in whole blood is to be de



lored, particularly when electrolyte balance is being studied, since the plasma or serum analysis gives a much more accurate picture. Serum or plasma has from 570 to 620 mg. per 100 ml. Calculated to milliequivalents per liter, this becomes from 97 to 106 meq. of Cl per liter for serum or plasma (see page 94). The only physiological factor which influences blood chloride materially is starvation. This is partly because of the lowered intake and partly because of the continued excretion of chloride until osmotic conditions force its retention. Many other factors have minor influences upon the chloride in normal individuals but none are of great importance.

Chloride usually rises in nephrosis, but in glomerulonephritis and in nephrosclerosis the chloride is usually unchanged. A low blood chloride is encountered in cases of persistent vomiting and the reason is obvious. Diarrhea leads to a similar result. In uremia, vomiting or diarrhea may be present, and here results a low blood chloride value.

The chloride level may be diminished in diabetes mellitus, particularly if acidosis is present. This is partly due to the diuresis, which washes out a considerable amount of chloride. Another cause is a shift of the chloride into the red blood cells because of the occurrence of acidosis. In Addison's disease the loss of sodium ions by way of the urine is accompanied by chloride loss and this results in a hypochloremia. Treatment with the cortical hormone or with sodium chloride tends to bring the level back to normal. Extensive burns also result in a low concentration of blood chloride, which is an indication for the replacement of this ion therapeutically.

## IODINE

The iodine content of blood is very minute but is considered extremely important, since fluctuations are due to changes in thyroid activity. Ordinarily, only "protein-bound iodine" of serum or plasma is determined, since this is felt to represent the thyroid hormone present in the circulating blood. The normal figures are given as 4 to 8  $\mu\text{g}$  (0.004-0.008 mg.) per 100 ml. of plasma or serum. The methods for this determination have been greatly improved, although they are still difficult and subject to a number of interferences. For example, there are certain medicaments in use which contain iodine and which may lead to spuriously high blood iodine values because they remain in the circulation for long periods of time, while others, containing mercury, interfere with the reaction and lead to low results. Nevertheless, under careful clinical control, and meticulous analytical conditions, valuable results are obtained. High values (e.g., 9 to 14  $\mu\text{g}$ .) indicate hyperthyroidism, and low values (e.g., 2 or 3  $\mu\text{g}$ .) are observed in cretinism and other types of hypothyroidism. The use of radioactive iodine in aiding in diagnosis is discussed on page 676.

## CARBON DIOXIDE COMBINING POWER

Since the ability of the plasma to combine with  $\text{CO}_2$  and form bicarbonate depends upon the quantity of available alkali present, the determination of the

$\text{CO}_2$  combining power, or  $\text{CO}_2$  capacity, of the plasma is a measure of the alkali reserve. The greater the amount of alkaline material present in the plasma, the greater the  $\text{CO}_2$  combining power. The results are expressed in volumes of  $\text{CO}_2$  combined by 100 ml. of plasma. The normal resting adult usually shows a range of from 50 to 70 volumes per cent. With mild acidosis and no pronounced symptoms, the range is likely to be from 41 to 50 volumes per cent. Here, ordinarily, the acidosis is well compensated; that is, there is no change in the pH of the blood. With moderately severe acidosis the alkali reserve shows greater depletion, and figures of from 31 to 40 volumes per cent are found. Now there are definite symptoms, and the acidosis may be uncompensated. With values below 30 volumes per cent, the blood almost always shows definite changes of pH toward the acid side and marked symptoms occur.

In alkalosis, the values, of course, are above 70 volumes per cent. However, the body does not have as efficient means of combating alkalosis as it does acidosis. Consequently an uncompensated condition is quite likely to occur when the values climb to about 75 volumes per cent, although values up to 125 volumes per cent do occur.

Most of the clinical conditions associated with disturbances in acid-base balance involve primary alkali deficit or primary alkali excess. The acidosis of diabetes, nephritis, and starvation come under the former heading, while pyloric obstruction, persistent vomiting, and alkali overdosage come under the category of primary alkali excess. Both of these can be determined by the estimation of the  $\text{CO}_2$  combining power. However, those conditions which involve primary  $\text{CO}_2$  excess and deficit cannot be properly evaluated by the determination of the  $\text{CO}_2$  combining power alone. Here pH determinations of the plasma are also essential.

### pH

It is apparent, from the foregoing discussion, that the  $\text{CO}_2$  combining power does not under all conditions give enough information regarding the acid-base balance, and the estimation of the pH of the blood plasma or serum is often highly desirable. For this purpose blood is drawn under oil to avoid gas exchange with the atmosphere. It is centrifuged and the serum or plasma, as the case may be, used for pH determination. This may be accomplished by either a colorimetric or an electrometric method.

### BILE PIGMENTS

The origin of bile pigments and their relation to jaundice have been taken up on page 236. It will be remembered that normally the liver liberates bilirubin from a colloidal complex and secretes it into the bile. In obstructive jaundice this liberation occurs, but the bile is prevented from flowing into the intestine. It finds its way into the blood and is excreted from the blood by the kidneys. In toxic and hemolytic jaundice there is no blockage of the bile, but the colloidal bilirubin is not broken down by the liver. Since this colloidal pigment cannot be eliminated, it continues to remain in the circulating blood.

Therefore there is the possibility of either crystalloidal or colloidal bilirubin being present in the blood under various circumstances.

To determine these substances there are several methods available. The icteric index is a very simple method for expressing the yellowish-orange color of blood serum. One must use a dry syringe to avoid hemolysis of the red cells and the patient should be in the postabsorptive state to avoid lipemia. Large amounts of carrots or other foods containing carotene should be avoided on the day preceding the test. The color is measured against a standard of potassium chromate and the result is expressed in units. The normal value is from 4 to 6 units. Values as high as 15 may occur without visible jaundice occurring. In severe cases amounts as high as 300 may be met. The icteric index does not differentiate between protein-bound and crystalloidal bilirubin.

The van den Bergh test is based upon the fact that bilirubin combines with Ehrlich's diazo reagent (diazobenzenesulfochloride) to form a red colored compound, azobilirubin (acetophenolazorubin). A direct test is one which is obtained by mixing serum with the diazo reagent and observing whether the color appears immediately or not. If it occurs within thirty seconds it is called an "immediate direct" reaction; if it does not occur within one minute but does occur later, it is termed a "delayed" reaction; and if it shows an immediate red reaction changing to violet on standing, it is called a "biphasic" reaction. The biphasic reaction is usually interpreted to be either the same as a direct immediate reaction or a mixture of the direct immediate and the direct delayed. The indirect test is made by first adding alcohol and saturated ammonium sulfate to the serum and then the diazo reagent. The color is then determined colorimetrically. This gives a color with both forms of bilirubin and is used for a quantitative determination of the total bilirubin in serum. The free or "crystalloidal" bilirubin reacts immediately in the van den Bergh test; that is, it gives an immediate direct reaction." The bound or "colloid" bilirubin gives a positive reaction only after enough time has elapsed to permit the splitting off of the pigment from this combination (delayed direct reaction), or after alcohol has acted upon it to dissolve out the bile pigment (indirect reaction). Consequently one finds that the direct reaction is obtained with blood serum from cases of obstructive jaundice, but in hemolytic and toxic jaundice there would be a delayed direct reaction. Unless one is desirous of obtaining an accurate quantitative measure of the bile pigments in serum, an icteric index determination, together with a qualitative examination of the urine for bilirubin and urobilinogen, will give the same information as a direct van den Bergh test.

The bile pigments are increased in blood serum as a result of (1) an increased rate of hemoglobin disintegration, (2) any condition resulting in diminished hepatic function, or (3) obstruction of the biliary passages. Increased hemoglobin destruction occurs in hemolytic jaundice, pernicious anemia, and in hemolytic infections. Decreased liver function is seen in acute yellow atrophy of the liver, in eclampsia, in typhoid fever, and in poisoning by chloroform, carbon tetrachloride, arsphenamine, and the heavy metals. In catarrhal jaundice, obstructive jaundice, and in diseases of the gall bladder there are duct obstructions which cause the bile to back up into the circulation.



Low values in the bilirubin content of serum are seen in the secondary anemias and also in aplastic anemia. The cells have a low color index. Hence when destroyed they yield little hemoglobin for bilirubin formation.

## ENZYMES

A few enzymes, present in plasma, have been found to show deviation from the normal under pathological conditions, and their estimation is used in diagnosis. Among these are the phosphatases, amylase, and lipase.

There are two general types of phosphatase which are of interest. The one most commonly studied is the one which has its optimal pH on the alkaline side, the "alkaline phosphatase." This phosphatase is the one concerned in bone formation. In general it increases in amount in the blood plasma in bone diseases. Several explanations have been offered to account for this. It may be the result of an overproduction of the enzyme in the bone to compensate for the lesion, or it may be a forced extrusion of the enzyme from the injured bone tissue. Another theory is that the bones' capacity for cellular activity is greater in the absence of normal bone synthesis, and with greater activity there is an increased formation of phosphatase. The unit generally employed is the Bodansky unit, which is that activity "equivalent to the actual or calculated liberation of 1 mg. of phosphorus as the phosphate ion during the first hour of incubation at 37° C. and pH 8.6, with the substrate containing sodium beta-glycerophosphate, hydrolysis not exceeding 10 per cent of the substrate." The normal range of values is from 1.5 to 4.0 units per 100 ml. for adults and from 5.0 to 13.0 units for children. Usually figures between 2 and 11 units have no pathological significance. In rickets, the values rise to from 20 to 30 units in mild cases, to 60 in severe cases, and up to as high as 190 units in very severe cases. In osteitis deformans (Paget's disease) the phosphatase is also very high, and increased concentrations are found in bone atrophy, osteomalacia, osteoporosis, and bone malignancy. Moderately increased values are found in hyperparathyroidism.

Since alkaline phosphatase is excreted into the bile by the normal liver, its level in the blood is related also to hepatic conditions. However, it is not a good diagnostic index unless a number of other factors are taken into consideration. Among these are the bone conditions just mentioned. Nevertheless, determination of alkaline phosphatase is of some value in aiding one to determine whether an obstruction to the common bile duct is present. Serum phosphatase values are almost always increased in jaundice due to mechanical obstruction.

The acid phosphatase, with an optimal activity at about pH 5.0, was first found in the urine of men. It apparently has its origin in the prostate gland, which was found to be extremely rich in this enzyme, and this led to the investigation of tumors of the prostate. Primary carcinoma of this gland, and metastases occurring in other tissues, contain acid phosphatase. The blood serum in cases of metastasis shows significant increases in the acid phosphatase content. No other prostate conditions lead to similar rises. However, determination of acid phosphatase is of limited value in diagnosing metastatic cancer of the

prostate. Prior to metastasis the serum level of acid phosphatase is apparently never elevated, but the rise with metastasis is not invariable; sometimes the level is not increased. Treatment of metastatic cancer of the prostate by methods which deprive the patient of androgen frequently results in a fall of the phosphatase, more or less parallel with the improvement in the patient's condition.

The amylase of the blood usually originates in the pancreas, although it may come from the salivary glands. It has been demonstrated experimentally and clinically that perforation of the gastroduodenal area may result in elevated serum amylase values. This is explained as a leakage of the pancreatic amylase into the peritoneal cavity with subsequent absorption of the enzyme from the peritoneal fluid. (Pemberton; Musgrove.) Serum amylase increases tremendously in cases of pancreatitis, but only for about forty-eight hours after the onset, whereas the blood lipase, which also rises in this condition, remains elevated for several days. Acute inflammation of the salivary glands causes moderate elevation of serum amylase, as does renal failure, with depressed excretion of amylase, and, as indicated above, perforation of the upper intestinal tract.

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## Chapter 23

# HORMONES

Hormones are substances manufactured by cells in minute amounts which produce characteristic physiological effects upon other cells, usually in some organ remote from the source of the hormone. They are exceedingly potent, and in this way they resemble vitamins. Indeed the only difference between these two classes of physiological agents is that vitamins are primarily elaborated by plants and are introduced into the body by way of the digestive tract, while hormones are formed by the body and are thrown directly into the blood stream. In many instances hormones are relatively ineffective when administered by mouth and, therefore, if given for medical purposes, are usually injected parenterally. Like the vitamins, some of the hormones have been synthesized, and, probably, they will also be shown to be parts of enzyme systems, at least in many cases. Plants, also, produce hormones which have definite effects upon plant physiology.

Hormones are, strictly speaking, stimulating substances (*hormaein*, to excite). However, some endocrine secretions inhibit functional activity, and these are designated "chalones."

### HORMONES OF THE GASTROINTESTINAL TRACT

In Chapter 10 the hormones of the gastrointestinal tract were discussed. It is only necessary to summarize their actions at this point.

Gastrin is produced by the pyloric mucosa, apparently stimulated to do so by substances present in or derived from food, or possibly by HCl. The mechanical stimulation caused by distention of the stomach also results in the production of gastrin. It is absorbed into the blood stream and is carried to the fundic cells and causes them to secrete HCl actively.

Secretin is formed by the intestinal mucosa and is liberated by the HCl present in the acid chyme. The secretin is carried by the blood stream to the pancreas, which it excites, and thus causes a flow of pancreatic juice. This occurs even if the nerves supplying the pancreas are cut and is therefore a true hormonal action. Further evidence in favor of this theory is the fact that if the blood from an animal, in the process of forming pancreatic juice due to the presence of HCl in the duodenum, is injected into the veins of a starving animal, the pancreas of the latter is stimulated to secrete. Secretin has been obtained in crystalline form and is very effective by the intravenous route. Melnby was of the opinion that secretin controlled the volume and bicarbonate content of pancreatic juice while the enzyme content was under the control of the vagus nerve. In 1943, however, another hormone was separated from the mucosal extract of the small intestine. This stimulates the secretion of juice, rich in enzymes, which is thus under both hormonal and nervous con-

trol. Secretin is the hormone which stimulates the production of fluid, low in enzymes but containing bicarbonate. The other hormone has been called "pancreozymin" and is present in the mucosa of the upper small intestine. It is thermostable and is not destroyed by acid but is by alkali. It may be separated from secretin from an alcohol solution of both, by precipitation of secretin by bile salts and precipitation of pancreozymin by saturation with sodium chloride (Harper and Raper). The release of pancreozymin is said to be brought about by the presence of any one of a variety of substances, including peptone, casein, dextrin, maltose, lactose, saline, and even distilled water. It is possible that similar stimulants may also be effective in causing the release of secretin. Secretin produces the same effects in man as it does in animals and it is being employed in tests for pancreatic function. (See page 660.) It probably also stimulates the flow of intestinal juice and is one of the factors which increase the secretion of bile by the liver. The flow of intestinal juice is also controlled by a hormone of the intestinal mucosa; namely, enterocrinin. It is distinct from secretin and stimulates the secretion of both fluid and enzymes by the intestinal mucosa. Various digestive products effect its release from the mucosa. Furthermore, extracts of intestinal mucosa contain a hormone which stimulates the contraction of the gall bladder. It is cholecystokinin and can be separated from secretin. Cholecystokinin is set free through the agency of many different substances. The most effective are fats, fatty acids, dilute HCl, and peptone.

Enterogastrone is a hormone which has been shown to be present in duodenal mucosa. Its formation is associated with the presence of fat in the duodenum. Its function is to inhibit gastric secretion and gastric motility. In other words, when fat reaches the duodenum it causes the secretion of enterogastrone which then slows up gastric digestion and motility. There is a diminution in the volume of juice secreted with a lower concentration of HCl and a smaller amount of pepsin. (Grossman.) The effect is to permit fat digestion to be more completely accomplished. From human urine a substance having similar effects has been isolated. It has been called "urogastrone" and it may be an excretory product of enterogastrone. It seems also to inhibit pancreatic secretion. Although there is good evidence for all of the humoral agents mentioned, some authorities feel that secretin and cholecystokinin are the only gastrointestinal hormones which have been fully established as such, according to strict physiological and biochemical standards.

## INSULIN

Insulin is the hormone elaborated by the islands of Langerhans of the pancreas. It is essential in carbohydrate metabolism. Its discovery, properties, and the theory for its mode of action have been described in Chapter 16. While its chief use is in the control of diabetes, it has been recommended in other conditions as well, including certain liver diseases. Possibly glucose alone would be just as useful in some of these, but probably the hepatic conditions are affected by this hormone in some specific way. It is also known that insulin hypoglycemia is accompanied by increased tonus and motility of the stomach, result-

g in hunger and sometimes increased appetite. Overdoses of insulin to produce a state of insulin shock are used in the treatment of certain mental disorders, often with beneficial effect.

Insulin is a protein with a molecular weight of about 6,000. Its isoelectric point is pH 5.3 to 5.36. It is inactivated by alkali which liberates ammonia and proteolytic enzymes which digest it. Its structure was discussed on page 116. It contains sulfur in the disulfide linkage,  $-S-S-$ . Attempts have been made to ascertain whether this or some other characteristic group is responsible for the activity of the hormone, but thus far it may only be said that certain disulfide groups and certain amino groups, both of which may be in the cystine

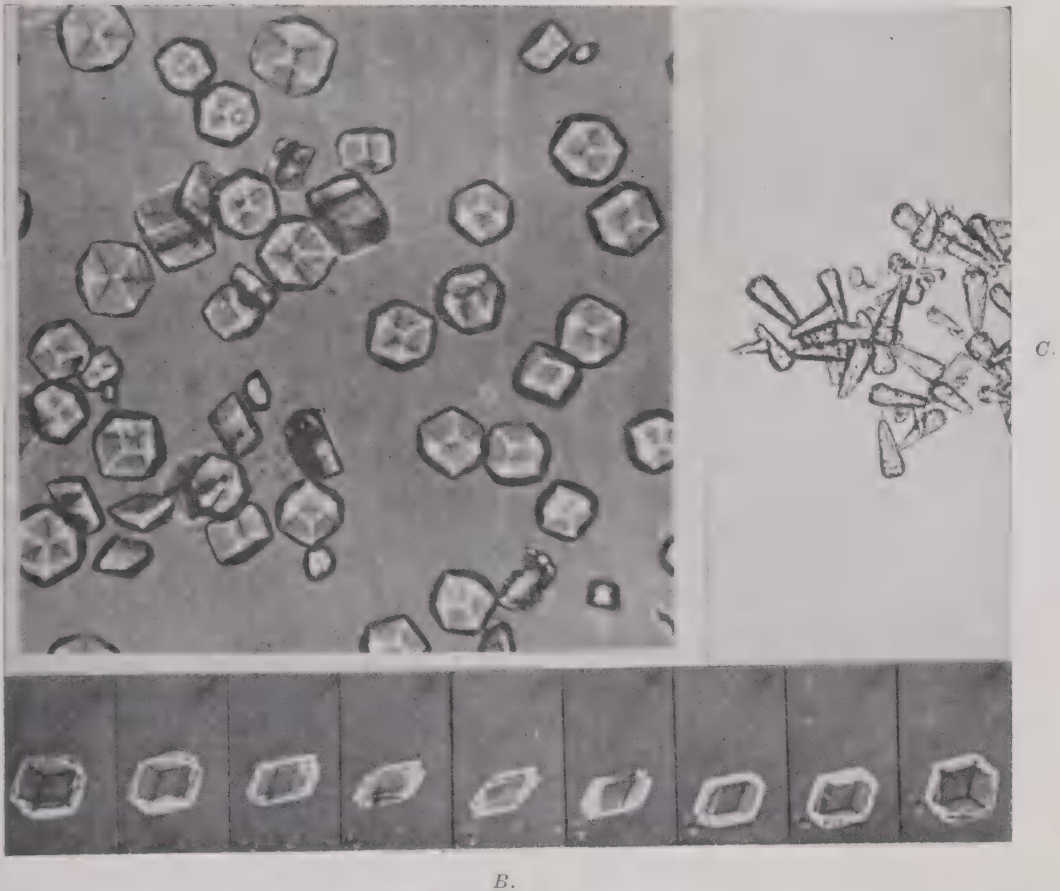


Fig 81.—Crystalline zinc insulin. *A*, Zinc insulin crystals formed at about pH 6; *B*, one of the same crystals, taken on motion picture film, as it rolls across the field; *C*, zinc insulin crystals formed at about pH 5. (From Scott, D. A.: *Endocrinology* 25: 437, 1939.)

portion, and certain phenol groups, possibly in tyrosine, are involved. Abel succeeded in crystallizing insulin, and now, by a more simplified procedure, crystalline insulin is produced commercially (Fig. 81). This is termed zinc insulin. Zinc is not present in the insulin molecule, but the addition of zinc (or cobalt, cadmium, or nickel) aids in the crystallizing process. Crystalline insulin is undoubtedly the natural hormone, not a derivative.

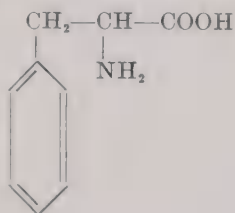
The potency of an insulin preparation is expressed in units. A unit is that amount of insulin required to reduce the blood sugar level of a normal rab-



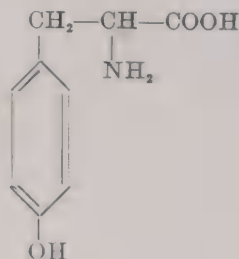
bit weighing 2 kilograms, which has been fasted for twenty-four hours, from 120 mg. to 45 mg. per 100 ml. in five hours. A more exact definition refers to a standard preparation of zinc insulin crystals kept by the National Institute for Medical Research in London. A unit is  $\frac{1}{22}$  mg. of this preparation. As sold for clinical purposes, insulin comes in concentrations designated "U-40," "U-80," and "U-100," which indicate the number of units per cubic centimeter. Protamine insulin and other types of slowly absorbed insulin are discussed on page 438. There is also present in insulin preparations a hyperglycemic factor (HGF), glucagon. (See page 435.)

## EPINEPHRINE

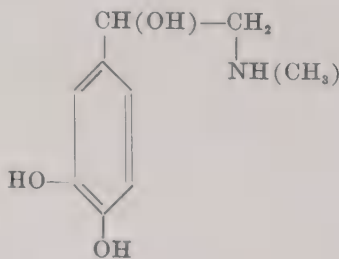
Epinephrine, adrenaline, or adrenine, is produced by the medulla of the adrenal glands. It was first isolated by Abel. Its structure has been determined, and it has been produced synthetically by Stoltz. Examination of its formula will reveal that it is closely related to tyrosine and phenylalanine. Experiments have been performed in which isotopically labeled phenylalanine was fed to animals and was shown to have been converted into epinephrine. The radioactive carbon was located in the carboxyl and  $\alpha$ -carbon positions of the amino acid, and the epinephrine recovered was found to bear a  $C^{14}$  at the position corresponding to the  $\alpha$ -carbon. (Gurin and Delluva.)



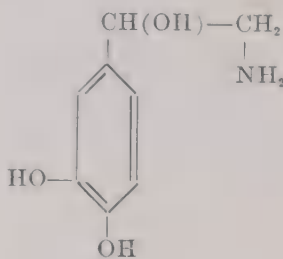
Phenylalanine



Tyrosine



Epinephrine



Nor-epinephrine

Since epinephrine possesses an asymmetric carbon, two stereoisomers are possible. The natural form is levorotatory and is fifteen times more potent than the dextrorotatory form. It is isolated from adrenal tissue by extraction with dilute acid, precipitation of protein with alcohol, and treatment of the filtrate with ammonium hydroxide. This precipitates the free base, a white substance which on exposure to the air is easily decomposed, and becomes pink, red, and finally brown.

Epinephrine is a "sympathomimetic" substance; that is, its effects resemble those produced by the stimulation of the sympathetic nervous system. These effects include constriction of the arterioles of the skin, mucous membranes, and splanchnic viscera, except the arterioles of the intestines. The latter dilate as do also the vessels of the muscles and the coronaries under the influence of adrenaline. The constricting effect has a greater influence on blood pressure than the dilating effect and, therefore, epinephrine generally produces a rise in blood pressure.

Epinephrine has an inhibitory effect upon the muscular tone of the stomach, intestine, bronchioles, and wall of the urinary bladder and upon the movements of the gastrointestinal tract. On the other hand, the muscles of the sphincter of the bladder and the intestinal sphincters contract. The effect of epinephrine on the uterus varies with the species and the state of the uterus; that is, whether it is gravid or not. In the human being, epinephrine causes it to contract only during pregnancy. The nonpregnant human uterus is inhibited. The pupil of the eye dilates as a result of contraction of the radiating fibers of the iris. It increases the rate and force of contractions of the heart.

It is now known that the adrenal medulla produces two hormones of very similar structure, epinephrine and norepinephrine. The presence of the latter in adrenal medullary extracts was suspected for a long time and was first demonstrated by pharmacological methods (von Euler. It has recently been isolated and characterized physiologically and chemically (Goldenberg; Tullar; Tainter). Norepinephrine, also called noradrenaline and L-arterenol, differs from epinephrine structurally in having an H in place of the methyl group. It also differs significantly in its pharmacological action. It is now believed that epinephrine raises blood pressure only because it increases cardiac output. It is an over-all vasodilator. Norepinephrine is a vasoconstrictor, except in the case of the coronary and intestinal arteries, and does not change the cardiac minute volume. They also differ in their effects upon the central nervous system, since norepinephrine produces less anxiety and discomfort.

Since these facts seem to be at variance with some of the statements made above, it should be remembered that the effects described were obtained with the mixture of the two substances ordinarily present in extracts of adrenal medulla commonly used. Such a mixture contains only about 10 to 20 per cent norepinephrine. Therefore the effects of commercial "epinephrine" are as mentioned above and are due chiefly to epinephrine. Norepinephrine also has a much less marked hyperglycemic action than epinephrine.

The injection of epinephrine causes hyperglycemia and glycosuria. This is because it increases liver glycogenolysis at first. It can in this way relieve the hypoglycemia produced by insulin. Its effect is, of course, greatest if there is a goodly store of glycogen in the liver. A similar effect upon muscle glycogen can also be brought about by epinephrine, resulting in increased lactic acid. This may be transported to the liver, where it is resynthesized to glycogen at the expense of muscle glycogen. According to Long, epinephrine may have a secondary effect, one, however, which leads also to hyperglycemia. The site of this action of epinephrine, and, to a lesser degree, of norepinephrine, is the anterior pituitary gland. This is stimulated by it to produce the adrenocorticotrophic hormone (see page 627). The latter is carried to the adrenal cortex, which it excites specifically to manufacture the adrenal cortical hor-

mones. As will be described later, some of these produce hyperglycemia. Thus, epinephrine, secreted by the adrenal medulla, indirectly affects the adrenal cortex. This action is slower than the primary action of epinephrine.

Epinephrine also has a "calorigenic" action. It increases oxygen consumption by from 15 to 40 per cent, but norepinephrine has a much weaker action. In man the basal metabolic rate rises promptly after the injection of 0.5 ml of the therapeutic 1:1,000 solution. This has no relation to the power of the thyroid to raise the basal metabolic rate. There are also stimulating effects upon circulation and respiration. It is thought that much of this action may be referred to the increased glycogenolysis, with resulting excess of glucose circulating and available for oxidation. This hormone has a number of other minor actions, including a stimulation of salivary and tear secretion, increase in the rapidity of blood clotting, and contraction of the spleen.

Solutions of epinephrine have wide application in medicine. The constriction of blood vessels and the shrinking of mucous membranes make it useful in stopping bleeding. Also in conjunction with local anesthetic solutions it tends to localize, intensify, and prolong their effects. It is used to relax the bronchioles in asthmatic attacks, and frequently combats allergic manifestations in a dramatic manner.

Despite the varied and definite physiological effects of its characteristic hormone, the adrenal medulla does not appear to be essential to life. Animals may be deprived of the adrenal medulla and get along quite well. No clinical syndrome is known which is attributable to a deficiency or disease of the adrenal medulla. Consequently its exact importance is really unknown. Cannon's theory that epinephrine is secreted in emergencies in order to raise the blood sugar to provide ready fuel for the necessary activity deserves consideration. If this is the true function of the adrenal medulla it would indicate why this gland is not absolutely essential to life although probably necessary for optimum physiological performance.

### ADRENAL CORTEX

Although epinephrine and the adrenal medulla are not indispensable, the same is not true of the cortex. An animal with both adrenals completely removed survives only one or two weeks. This is almost certainly due to the absence of adrenal cortical tissue.

The chief symptoms which occur in such animals are:

1. A disturbance of the electrolyte and water balance. There is increased excretion of sodium, chloride, and water and retention of potassium. As a result, the sodium and chloride content of the blood decreases, potassium increases, and there is hemoconcentration. Although an excess of potassium is generally considered deleterious, it is not certain that death results from potassium poisoning in adrenal deficiency.

2. The urea content of the blood rises. This may be due in part to a decrease in renal blood flow; in part, to decreased kidney function.



3. There is great muscular weakness. This is probably secondary to the effects upon carbohydrate metabolism and upon the salt and water balance.

4. There is a decrease in liver glycogen, with hypoglycemia and a greater sensitivity toward insulin. It is generally believed that these disturbances in carbohydrate metabolism result from a diminished utilization of carbohydrate and a decreased formation of sugar from proteins (gluconeogenesis). The "Long animal" strikingly illustrates the general effect. This is an animal with both the pancreas and adrenals removed. The pancreatic diabetes is not as severe as the absence of the adrenal cortex as when it is present, thus demonstrating the opposing effects of insulin and the cortical carbohydrate hormone. Moreover, the average length of life after operation is about three times as great for those animals that have been both adrenalectomized and depancreatized as compared with those only depancreatized. The administration of extracts of adrenal cortex to normal animals results in an increase in blood sugar, liver glycogen, and muscle glycogen (Britton and Silvette).

Along with a decreased formation of sugar from proteins, there occurs a decrease in the quantity of protein catabolized. As would be expected, the injection of cortical hormones into normal fasting animals *increases* the rate of protein breakdown. The adrenal cortical hormone must accelerate protein metabolism. Consequently the administration of the hormones of this gland to normal young animal will retard its growth because they specifically stimulate the breakdown of protein tissues.

5. A reduced ability to withstand stress, such as cold, or mechanical or chemical shock. This is also probably referable to the primary effects upon carbohydrate and electrolyte metabolism.

6. There is an effect upon lymphoid structures. (See page 613.)

7. Retardation of growth occurs if adrenalectomy is performed on a young animal. This undoubtedly indicates some basic action of the adrenal cortex upon all cells of the body, while the more specific symptoms previously mentioned indicate the most prominent effects on individual organs.

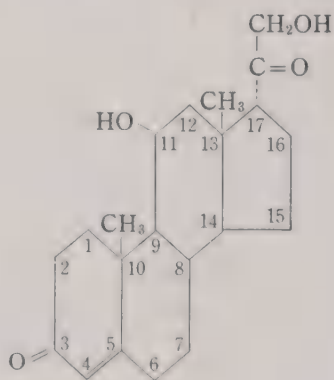
Administration of cortical extracts will remedy all of these symptoms, change the chemical picture practically to normal, and extend the life of the adrenalectomized animals for months. The production of potent extracts suitable for use in human beings has resulted from these experiments. We owe our knowledge, and many patients their lives, to the brilliant work of Rogoff, Hartman, Swingle, Grollman, and their co-workers.

From an organic chemical standpoint, Kendall, Reichstein, and Wintermeyer have been particularly active and successful. They have isolated twenty-eight crystalline steroids from this tissue. The structure and configuration of these steroids are known in detail, and some have been synthesized. Six or seven of them have been found to be active physiologically in being able to prolong the life of adrenalectomized animals or in preventing or curing single symptoms present in such animals. They are often grouped into two classes from a functional standpoint: the "mineralocorticoids," with a predominant action

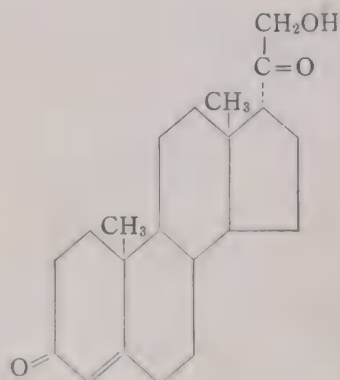
upon electrolyte metabolism, and the "glucocorticoids," with a pronounced effect upon carbohydrate metabolism.

One of the most active substances obtained from the gland is corticosterone, an example of the glucocorticoids. Another of the natural steroids present possessing one less oxygen, termed desoxycorticosterone, one of the mineralocorticoids, has been prepared synthetically and is widely used therapeutically. It is much more powerful than corticosterone in its effect upon electrolyte balance, although it does not have a favorable effect on carbohydrate metabolism. Recently "compound E," 17-hydroxy-11-dehydrocorticosterone (cortisone), has had a dramatic impact upon medicine. It was first found to have a remarkably beneficial action upon the severe symptoms of rheumatoid arthritis, and later upon other pathological conditions, and it may prove to have wide applications. (See also page 679.) (Hench.) "Compound F," 17-hydroxycorticosterone, is also known to be secreted by the adrenal cortex and may prove to be of great importance.

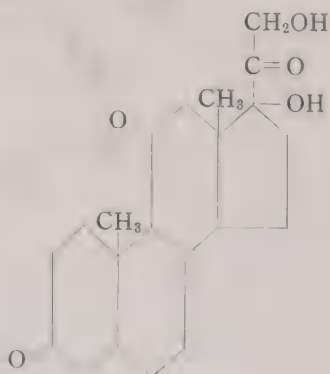
Their formulas are as follows:



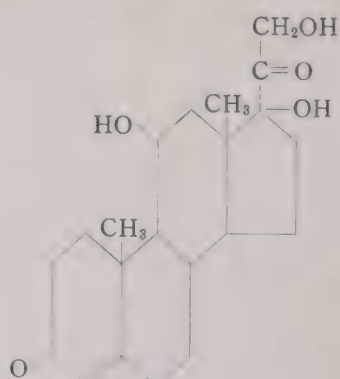
Corticosterone  
( $\Delta^4$ -Pregnene-3,20-dione-11,21-diol)



11-Desoxycorticosterone  
( $\Delta^4$ -Pregnene-3,20-dione-21-ol)



17-Hydroxy-11-dehydrocorticosterone  
( $\Delta^4$ -Pregnene-3,11,20-trione-17[ $\beta$ ],21-diol)



17-Hydroxycorticosterone  
( $\Delta^4$ -Pregnene-3,20-dione-11,17[ $\beta$ ],21-triol)

In man the syndrome of Addison's disease represents a hypofunction of the adrenal cortex. Many of the symptoms resemble those of adrenalectomized animals. These were described by the discoverer of this syndrome, Thomas

Addison, in 1855 in these words: "The leading and characteristic features of the morbid state to which I would direct attention are anemia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change of color in the skin, occurring in connection with a diseased condition of the 'supra-renal' capsules." To these symptoms today would be added low blood pressure, lowered basal metabolic rate, sub-normal temperature, and a disturbance in the water and electrolyte balance.



Fig. 82.—Addison's disease. Universal pigmentation; advanced asthenia. Note the marked pigmentation of the face, hands, feet, and nipples. Post-mortem examination showed perculosis of the adrenals. (Courtesy Dr. Robert T. Frank, New York, N. Y.; case from the office of Dr. B. S. Oppenheimer.)

This includes a loss of  $\text{Na}^+$  and  $\text{Cl}^-$ , an increase in  $\text{K}^+$ , and a loss of body water. There is also a hypoglycemia which indicates that there is a profound effect upon carbohydrate metabolism. The kidneys are also affected, resulting in a urea retention. The pigmentation occurs in those locations where the normal pigmentation is greatest. Frequently the face and neck and backs of the hands are



so deeply bronzed as to cause the afflicted individual to look like a mulatto (Fig. 82).

Treatment with extracts of the adrenal cortex has been very successful. This began in about 1929. Since this is a substitution therapy, as most endocrine therapy is, the constant administration of potent extracts is essential. The administration of the natural cortical extracts to patients suffering from Addison's disease resulted in marked improvement in many cases. Soon after cortisone became available, it was found that a small daily dose of it, plus a few milligrams of desoxycorticosterone, controlled the symptoms of Addison's disease as efficiently as glandular extracts. Cortisone is now made by partial synthesis from naturally occurring steroidal starting materials. Total synthesis of cortisone has also been accomplished. (Sarett.)

Loeb and his co-workers showed that the administration of sodium chloride alone was of immense value to sufferers from Addison's disease. It corrected the electrolyte and water imbalance and led to decided improvement of the clinical symptoms. The usual treatment today is to combine the salt and hormone medication. Sodium chloride is fed or injected in fairly large amounts (from 7 to 20 Gm. per day in addition to that in the diet), often along with sodium citrate. The citrate ion is oxidized away, leaving a preponderance of sodium ions. Before desoxycorticosterone or cortisone was available, many patients were brought out of severe crises of the disease by the parenteral administration of physiological solutions of sodium chloride. Today the feeding of sodium salts permits the patient to get along on a lower dosage of the hormone, or even with none at all, for long periods of time. At the same time the restriction of potassium in the diet is distinctly helpful.

The administration of desoxycorticosterone, usually as the acetate, affects almost exclusively electrolyte and water metabolism. It causes retention of sodium and restores the blood sodium level to normal. A retention of water is brought about, thus increasing the volume of the blood plasma and the interstitial fluid. It increases the elimination of potassium, resulting in a reduction of serum potassium to normal and even subnormal levels. Renal function is restored, so that urea and other nonprotein nitrogenous constituents are excreted, leading to a diminution in the blood N.P.N. There is also a decrease in the total protein, calcium, and cholesterol of the blood serum, probably as the result of the retention of water and dilution of these constituents (Fig. 83).

Desoxycorticosterone acetate is sometimes administered in the form of a pellet of the crystals implanted beneath the surface of the skin. These weigh between 100 and 150 mg. each. The dose necessary to maintain the patient at a normal weight must first be ascertained by intramuscular injection. Then one pellet is implanted for every 0.4 to 0.5 mg. required per day. The hormone is absorbed at a slow and uniform rate. This obviates the necessity of daily injections. Since desoxycorticosterone does not correct the defect in carbohydrate metabolism, hypoglycemia may occur. It is therefore necessary that the diet contain large amounts of readily available carbohydrate. It should also be mentioned that the hormone does not have a very constant or complete effect in banishing the pigmentation of the skin in Addison's disease.

Whether any single adrenal-cortical steroid can fully substitute for the natural secretion of the adrenal cortex has not been answered to the complete satisfaction of all of the investigators in the field. It has been claimed that cortisone can be used successfully in replacement therapy of individuals who have been bilaterally adrenalectomized. Furthermore, it has been suggested that 17-hydroxycorticosterone may be the only natural secretory product of the adrenal cortices. (Conn.) However, Ingle finds that neither cortisone or 17-hydroxycorticosterone can fully replace adrenal extract in sustaining the ability of the adrenalectomized rat to perform work. In fact, the amorphous fraction of adrenal cortex extracts is more powerful in this respect than any single, known steroid.

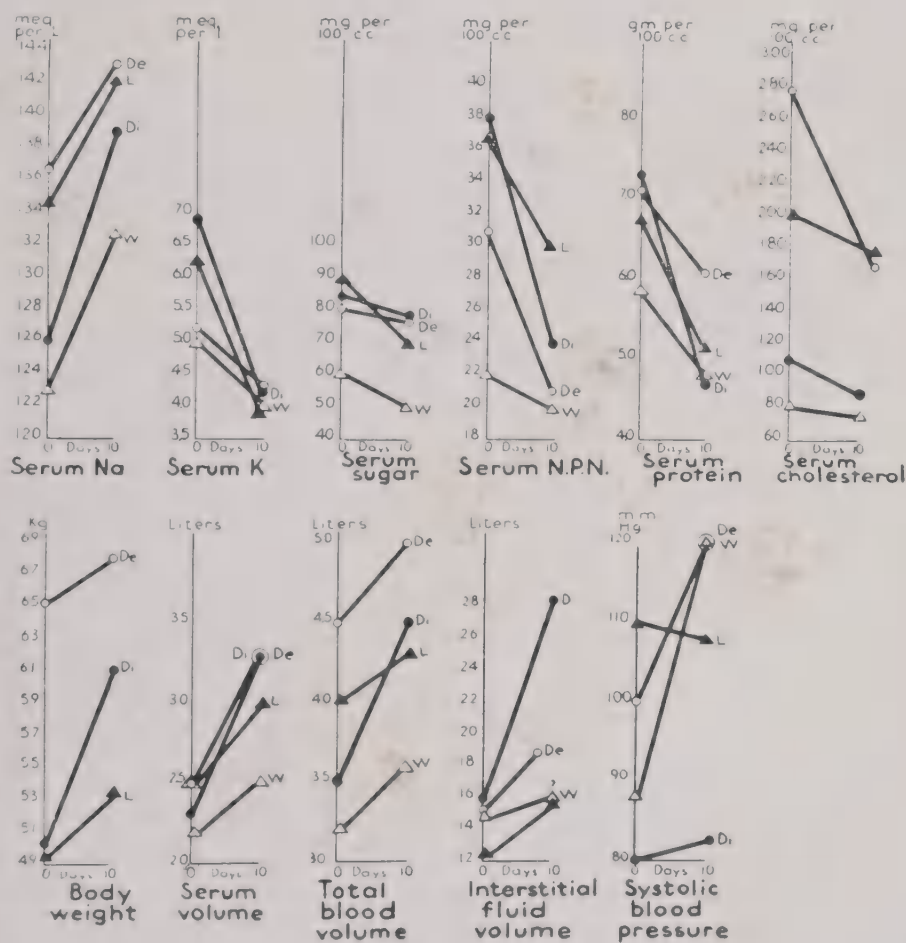


Fig. 83.—Summary of the effects of desoxycorticosterone propionate observed after ten days' treatment of a group of patients with Addison's disease maintained on a standard regimen. The serum sodium rose and the potassium fell. The serum N.P.N. fell as a result of increased excretion of urea. The serum protein concentration fell because of an increased excretion of all body fluids, which also accounts for the rise in body weight, and the improvement in blood pressure. Serum sugar did not rise but, if anything, fell, perhaps because of the treatment. (From Ferrebee, J. W., Ragan, C., Atchley, D. W., and Loeb, R. F.: *J. A. M. A.* 1725, 1939.)

The adrenal cortex has a high concentration of both cholesterol and ascorbic acid. In fact, vitamin C was first isolated from adrenal cortical tissue by Szent-Györgyi. However, since the glands are extremely small, the actual amounts

of cholesterol and ascorbic acid are not large in comparison with those present in other tissues. Apparently the cholesterol is a precursor of the steroid hormones elaborated by the cortex. The injection of adrenocorticotrophic hormones, which will be described later, stimulates the adrenal cortex to secrete these hormones. When this occurs, both the cholesterol and ascorbic acid contents of the adrenal cortex decrease in concentration. (Sayers.) The function of ascorbic acid in the adrenals is unknown. It may be related to cellular respiration and metabolic rate. In stress, there is a greater requirement and utilization of ascorbic acid, and this seems to be related to the increase in adrenocortical function, which is seen in stressful situations. (Pirani.) It will also be remembered that in scurvy, vitamin C avitaminosis and arthritic symptoms are seen. Cortisone prevents these from occurring in the guinea pig, whereas desoxycorticosterone aggravates such symptoms. Hence, it is possible that ascorbic acid is a necessary component of the oxidation-reduction system which produces the oxy type of adrenal hormones, such as cortisone. (Hughes.) Another vitamin which is involved in the production of the adrenal steroids is pantothenic acid. Animals on a diet deficient in this factor develop lesions of the adrenal cortex and fail to have satisfactory adrenocortical function. Apparently pantothenic acid acts as a catalyst in the production of these hormones. (Cowgill.)

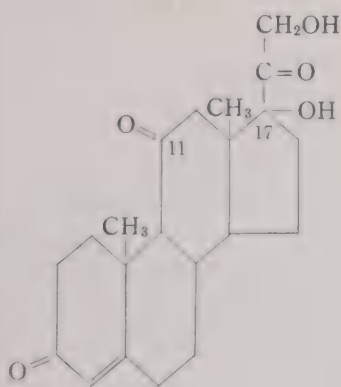
The adrenal-cortical secretion is partly under the control of the adrenocorticotrophic hormone of the anterior pituitary gland. It is also under the influence of the autonomic nervous system, the stimulation of which releases epinephrine. This hormone causes an increased activity of the adrenal cortex, as mentioned previously, by stimulation of the anterior pituitary to produce ACTH, which in turn stimulates the adrenal cortex. (McDermitt.) However, the various types of stresses which are associated with increased activity of the sympathetic nervous system (pain, trauma, heat, cold, burns, hemorrhage, etc.) all evoke the secretion of adrenal-cortical hormones, and these cause the manifold physiological effects described previously. The relationship between the anterior pituitary gland and the adrenal cortex will be discussed further in a subsequent portion of this chapter.

**The Adrenogenital Syndrome.**—The adrenal cortex seems to be the origin of many instances of abnormal sexual changes. Sometimes definite tumors of the cortical tissue have been found to account for these changes, but other cases of tumors of the same types of cells are not followed by these symptoms, and most of the cases have shown at autopsy glands which were either grossly normal or more or less hyperplastic (i.e., increased in amount of tissue). The sexual changes are mostly toward the masculine side. If they occur very early in life, they may produce, in the female, *pseudohermaphrodisim*, that is, the external genitals become masculinized to such an extent that they resemble male organs. There are also changes in the secondary sexual characteristics. In very young girls the sex organs and the breasts may assume adult size and appearance and menstruation may occur. In adult women masculinization occurs—the voice

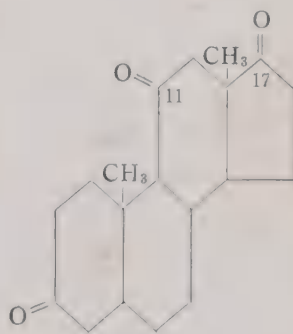


opens, the breasts atrophy, menstruation ceases, the pubic hair changes to the male pattern, and a beard may grow on the face. Boys likewise show precocious sexual development, and sometimes both boys and girls acquire unusual musculature. In the adult male this condition is rather rare but when it occurs there is sometimes an exaggerated male sexual development and desire, sometimes a feminization, but frequently no sexual changes whatever.

The cause of this syndrome is unknown. Some maintain that it is due to the overfunctioning of certain cortical cells which secrete a masculinizing, or androgenic, substance. Others suggest that there is present in the cortex "bisexual" glandular tissue which is controlled by the pituitary gland and can secrete both androgens and estrogens. (Vines.) In the fetus, shortly after the differentiation of the gonads has been completed, there is an androgenic phase." If this is continued too long in the female, it may produce those masculinizations described for the young subject, including pseudomaphrodisia. Adolescent virilism is believed to result from later fetal masculinization without affecting the gonad and remaining latent until puberty. It is definitely known that the adrenal cortex can elaborate both androgens and estrogens. Reichstein and Shoppee isolated from beef adrenals adrenosterone which has about one-fifth the androgenic potency of androsterone, a "male" sex hormone to be discussed later. Its relationship to one of the typical cortical steroids, 11-dehydro-17-hydroxy corticosterone is shown below. The latter is one of the hormones capable of supporting life in an adrenalectomized animal.



11-Dehydro-17-hydroxycorticosterone  
( $\Delta^4$ -Pregnene-3,11,20-trione-17[ $\beta$ ],21-diol)



Adrenosterone  
( $\Delta^4$ -Androstene-3,11,17-trione)

The urine of patients suffering from this syndrome frequently, but not always, contains increased amounts of androgenic substances, the "17-keto-steroids" (see page 637). Cortisone frequently has a beneficial effect.

**Effect of Adrenal Cortex on Lymphoid Tissue.**—An additional function of the adrenal cortex has recently been shown by White, Dougherty, and Chase. They had first been found that there was an inverse ratio between the size of the adrenal cortex and that of the thymus gland. In adrenal cortical deficiency there is hypertrophy of the thymus, and atrophy of the thymus follows increased

activity of the adrenal cortex. (Selye.) When cortical hormones are injected into normal animals, a rapid decrease in the number of circulating lymphocytes occurs. This is followed by a rise in total serum protein and particularly in serum globulin. The adrenal cortical hormone, corticosterone, stimulates a disintegration of lymphocytes in the lymphoid tissues and in the blood. This "lymphocytolysis" releases the contents of these lymphocytes into the body fluids and ultimately into the blood stream. Extracts of normal lymphocytes have been shown to contain a substance similar to, if not identical with, the  $\gamma$ -globulin of the blood. In animals previously immunized to antigenic substances before treatment with cortical hormones, the concentration of the specific antibodies rises faster and to higher levels than in those animals not receiving the hormone. This suggested that the lymphocyte serves as a storehouse for labelled  $\gamma$ -globulin, or immune bodies. Experiments using lymphocyte extracts from immunized animals showed a concentration of immune bodies in the lymph tissues greater than that of the serum. Disintegration of the lymphocytes after administration of adrenal cortical extract caused a rise in serum antibodies. Similarly, if immunized animals are exposed to any of the various stresses which call forth the secretion of adrenocortical hormone, they will likewise have higher concentrations of antibodies in their sera. The same is true of the injection of adrenocortical hormone.

Therefore, it would seem that the lymphoid tissue, in general, and lymphocytes in particular, act as a storehouse for antibodies and that this tissue is under the control of the adrenal cortical hormone for the disintegration of lymphocytes and the ultimate release of antibodies into the blood stream.

### THYROID GLAND

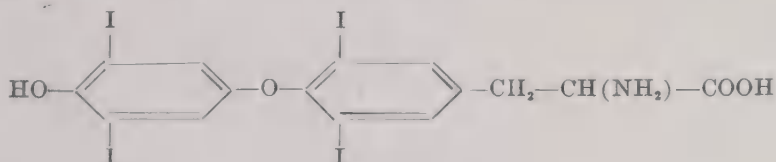
The early work on the thyroid gland was complicated by the fact that investigators did not know of the existence of the parathyroids. The thyroid consists of two lobes, one on each side of the trachea just below and anterior to the larynx, with a connecting "isthmus." It weighs about 30 grams. There are usually four parathyroids, two at or near the dorsal surface of each lobe of the thyroid, but there may be fewer than four, or as many as eight, and their location is similarly variable. They are very small and are closely connected with, or imbedded in, the thyroid tissue. Consequently, when the thyroids were removed for experimental purposes, all of the parathyroids were sometimes removed at the same time. This resulted in a loss of both the thyroid and parathyroid secretions. In 1891 the importance of the parathyroids was recognized and the confusion was dispelled.

The thyroid gland is composed of a large number of tiny closed vesicles lined with epithelial cells and filled with a colloidal material commonly called "colloid." It is richly supplied with blood vessels. The colloid material, which probably contains the hormone secreted by the cells, is believed to be reabsorbed by these cells and to be secreted into the blood stream.

Any enlargement of the thyroid gland, except of an inflammatory or malignant character, is termed a *goiter*. Simple goiters are not accompanied

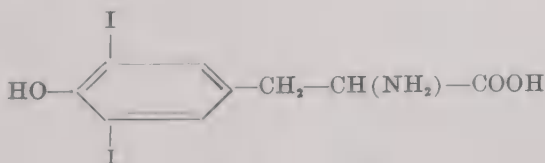
constitutional symptoms. They are said to occur more frequently in certain regions of the earth—those far from the ocean or shielded from sea breezes by high mountains. Sea water contains relatively high concentrations of iodine and it is generally stated that the lack of iodine in the drinking water and foods in these regions is associated with the prevalence of this condition. (See page 623.) Other types of goiters are seen both in hypothyroidism (cretinism and myxedema) and hyperthyroidism (exophthalmic goiter and toxic adenoma).

The hormone secreted by the gland contains iodine. In fact, more than 99 per cent of all the iodine in the body is concentrated in the thyroid. Apparently the thyroid is a sort of trap for excess iodine. A gradient of iodine concentration is established so that the concentration of iodine in the thyroid is many times that of the circulating blood. Normally the blood contains about 0.5  $\mu\text{g.}$  per 100 ml., and the thyroid tissue can hold about 10  $\mu\text{g.}$  per 100 Gm. This gradient of 20:1 is increased to several hundred to one by stimulation of the thyroid by thyrotrophic hormone (see page 627), by iodine deficiency, or in Graves' disease. This concentrated iodine is probably within the cells. The relationship of iodine to the thyroid has long been known, and iodine has been used in the treatment of simple goiter for over a century. It is more effective in preventing simple goiter than in curing it. There is present in the gland an iodized protein called thyroglobulin or iodothyroglobulin. This has marked physiological properties. In 1919 Kendall obtained a crystalline substance from thyroid which is highly active. He called it thyroxine. Harington and Barger established its chemical formula as:



Thyroxine

It will be noted that thyroxine is closely related to tyrosine. In fact, much of the iodine present in the gland (about 70 per cent) is present as an even lesser relative to tyrosine, namely, diiodotyrosine, which has little physiological activity, and some monoiodotyrosine. (Fink and Fink.)

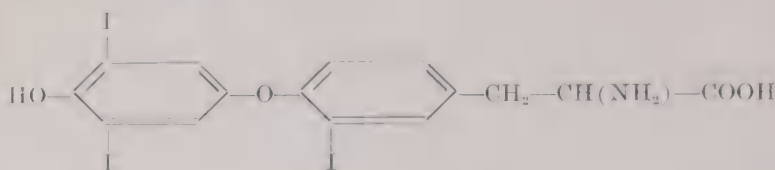


Diiodotyrosine

It is believed that diiodotyrosine is the precursor of thyroxine. This is on the basis of experiments in which radioactive iodine was used in both injection and tissue slice experiments. The evidence indicated that the tagged iodine went through the diiodotyrosine stage before being incorporated into thyroxine. (Taurog and Chaikoff.)



Gross and Pitt-Rivers have isolated from thyroid tissue 3,5,3'-triiodothyronine. (Thyronine is the iodine-free skeleton of thyroxine.) This has five times the physiological activity of thyroxine and is present in relatively small amounts.



3,5,3'-Triiodothyronine

It is suggested that this compound is the peripheral thyroid hormone and that thyroxine is its precursor. Apparently the iodination of tyrosine, with the formation of diiodotyrosine, occurs after iodine, in the form of iodide, is taken up, concentrated from thirty to several hundred times by the thyroid gland, converted to nascent iodine, and incorporated in the thyroglobulin molecule. The gland then converts diiodotyrosine into thyroxine, which, in turn, is changed to triiodothyronine, perhaps in the peripheral tissues. The circulating blood contains thyroxine and small amounts of triiodothyronine.

It is possible that neither thyroxine nor triiodothyronine is the true thyroid hormone. Some authorities maintain that it is a much larger molecule, such as thyroglobulin or a peptide complex containing one or more of the iodine-containing compounds. Thyroglobulin has a greater activity than thyroxine in proportion to its iodine content. The estimation of protein-bound iodine in blood is considered a good measure of thyroid function.

Two functions are attributed to the thyroid gland. It has a profound effect upon the growth and development of the body, and it has a stimulating effect upon total metabolism.

In young animals, removal of the thyroids, without disturbance of the parathyroids, results in an arrest of growth. In the human being a similar effect is seen when the thyroid is atrophied at birth. The resulting condition is termed *cretinism*. Cretins are abnormal dwarfs. They frequently have bowed legs, thick skin, and coarse hair. Although they may grow to adulthood, they do not develop mentally. The sex organs remain small and the abdomen becomes distended. In the adult the clinical condition of hypothyroidism is known as *myxedema*. The symptoms include changes in appearance of the patient—the skin becomes thick and puffy, and there tend to be swellings under the eyes. This is said to be due to the deposition in the skin of additional protein material. Although general intelligence is not impaired, the patient is slower in thinking, as well as in movements. There is a low B.M.R., an increased deposition of fat, and high blood cholesterol. The sexual functions are usually diminished.

In cretinism, treatment with thyroid preparations must be begun very early to have any beneficial effect, and the treatment must be continued as long as the thyroid gland fails to function. Myxedema yields to treatment quite dramatically. Whether thyroid gland or thyroxine is given, the patient is usually brought back to a normal state (Figs. 84 and 85).

The influence of the thyroid upon metabolism is more evident in cases of hyperthyroidism. In exophthalmic goiter the basal metabolic rate increases considerably above the normal figures—80 per cent above normal is not unusual. Consequently more food is consumed, in spite of which a loss of weight usually occurs. The high metabolic rate demands a more rapid glycogenolysis, resulting in a mild hyperglycemia, and sometimes a small amount of glucose in the urine. The patient often feels hot because of the increased heat production. Other symptoms include protrusion of the eyeball and dilated pupil, mental excitement, and irritability. There is also an accelerated pulse, cardiac dilatation, and other cardiac effects. The hormone appears to act directly upon heart



Fig. 84.—Cretinism. A, Age 7 weeks. Typical cretin: protruding tongue, coarse hair, umbilical hernia, dry skin, cool extremities, subnormal temperature, mentally dull and inactive, wailing cries, indifferent feeder. Desiccated thyroid medication begun.

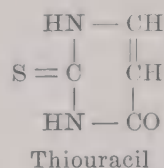
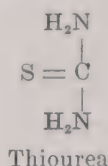
B, Age, 10 weeks. Striking improvement in three weeks. Temperature normal; nursing satisfactorily; extremities warm. Note changed facial expression.

C, Age, 5½ years. Normal mental and physical progress.

D, Age, 9 years. Weight, 61 pounds, 12 ounces; height, 49½ inches. Normal mentality. Serve school report card with high rating in basic subjects, particularly mathematics, in which subthyroid patients are usually defective. Thyroid therapy has been continued through- (From Kerley, C. G.: Arch. Pediat. 57: 432, 1940.)

muscle to produce this more rapid rate. Tissues from an animal which has had thyroid removed have a decreased metabolism; addition of thyroglobulin increases it, but thyroxine does not. If the tissues derived from an animal which has been fed thyroid are tested, they also are found to have an increased metabolic rate. Hyperthyroidism may be combated by surgical removal of

some of the overactive thyroid tissue. A chemical method of accomplishing the same effect is by the administration of thiourea or thiouracil (or of large doses of sulfonamides).



These antithyroid drugs cause inhibition of the secretion of the hormone by the gland, probably by preventing the coupling of two di-iodotyrosine molecules to form thyroxine. (Dempsey and Astwood.) They do not interfere with the action of the hormone if it is administered simultaneously. The 6-propyl derivative of thiouracil is five times as potent as thiouracil itself and is much



Fig. 85.—Spontaneous myxedema before and after treatment with thyroid. (Courtesy Dr. Ephraim Shorr; case of Dr. David P. Barr.)

less toxic. Substances of this type are said to be present in certain foods, the excessive ingestion of which causes simple goiter. Since they inhibit the formation of the thyroid hormone, the pituitary gland secretes greater quantities of the thyrotrophic factor, and a compensatory hypertrophy of the thyroid results. (See page 627.) Among the “goitrogenic” foods are cabbage and related vegetables, turnips, soybeans, peanuts, and the seeds of mustard. An antithyroid factor has been isolated from turnips and other goitrogenic vegetables. Evidence points to its identity as 1-5-vinyl-2-thio-oxazolidone. (Astwood.)

Thyroid preparations are often used to increase the basal metabolic rate. This may be necessary in certain types of obesity, especially if hypothyroidism

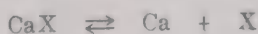


s present. Because of the effects upon the circulation, this must necessarily be done with caution, for thyroid administration is quite dangerous in many cases. Not only thyroglobulin and thyroxine possess this calorigenic action, but also other closely related substances. These must have as a minimum requirement two atoms of iodine attached to a tyrosine nucleus. Simply iodinating a protein will give it thyroidlike properties.

## THE PARATHYROID GLANDS

The close anatomical relationship between the parathyroid and the thyroid glands has been mentioned. When both are removed experimentally, the animals develop severe tetany and often die. This particular symptom is due to the loss of the parathyroids. Since tetany may also be produced by other methods, this type is called *tetania parathyreopriva* or *parathyroid tetany*. If the parathyroids are removed in a human being, either accidentally when the surgeon is excising thyroid tissue or in the case of a malignancy, tetany may occur. This is a danger even if a considerable proportion of parathyroid tissue remains, since this remaining tissue may not immediately produce a sufficient amount of the hormone. MacCallum and Voegtlin showed, in 1908, that parathyroidectomy was followed by a diminution in the calcium content of the blood serum and a rise in the phosphorus. The calcium and phosphorus excretion in the urine are both diminished. The tetanic symptoms seem to be due to a low calcium content of the serum because the intravenous injection of calcium salts relieves them very quickly. It is now well established that the parathyroid hormone's role is that of a regulator of the levels of these two elements. Administration of this hormone, obtained by Collip from beef parathyroids, is also effective in parathyroid deficiency. It not only relieves the tetany within a few hours, but also brings the calcium and phosphorus blood values back to normal.

The parathyroid hormone, parathormone, is protein in nature, since it can be digested by proteolytic enzymes. Probably two proteins are present, one having a molecular weight of about 20,000 and another of about 500,000 to 1,000,000. (Ross and Wood.) Administration of it (1) raises the blood calcium and lowers blood phosphorus, (2) increases the elimination of both in the urine, (3) causes the migration of calcium from the bones if this element is not available in sufficient amounts in the food, and (4) increases the phosphatase activity of the serum. There are several theories to account for these effects, but no general agreement has been reached. Some believe that the hormone acts directly on the bones, stimulating cellular activity and the removal of calcium. Greenwald suggests that the hormone is necessary for the formation of very slightly dissociated calcium compounds in blood. It may even be the very substance, X, which unites with calcium to form this organic calcium compound CaX. There is an equilibrium between the small amount of inorganic ionized calcium, the organic calcium, and the solid calcium of the bone. Administration of parathyroid extract to a normal individual displaces the equilibrium



to the left. This results in a decreased concentration of calcium ions. Hence the inorganic calcium equilibrium is similarly shifted to the left



and bone loses its calcium phosphate. This results in an increased elimination of these ions by way of the kidney. According to Albright, parathormone causes the secretion of phosphate in the urine, and this results in a lowered level of phosphate in the blood. In order to maintain a normal Ca-P solubility product in the plasma, calcium is mobilized from the bones, and a hypercalcemia results. This is the cause of the increased secretion of calcium in the urine.

The normal level of calcium in blood serum is about 9.5 to 11 mg. per 100 ml.; in a completely parathyroidectomized person it falls to from 5 to 7 mg. All of the symptoms of parathyroid deprivation may be referred to this low serum calcium. The object of therapy, therefore, is to bring the calcium level up to normal but not much higher, since hypercalcemia is as dangerous to life as is hypocalcemia. At a level of about 7.5 to 9.0 mg. the threshold is found; above this, calcium is excreted in the urine. Consequently, if the calcium level can be raised until it is just excreted in the urine, a safe level will probably be attained. Such a procedure would obviate the necessity of frequent blood examinations. Clinicians recommend raising the blood calcium level by administering one of the vitamin D compounds, such as vitamin D<sub>2</sub> (calciferol) or dihydrotachysterol, until calcium appears in the urine. This can be quickly ascertained by using the Sulkowitch reagent, a buffered solution of oxalates, which, when added to urine, produces an immediate precipitate of calcium oxalate, varying in density with the amount of calcium present. It is, of course, necessary to have the patient on a diet containing sufficient calcium for his needs.

Hyperparathyroidism may occur in man. It is known as osteitis fibrosa cystica or von Recklinghausen's disease. A high blood calcium, low phosphorus, and high plasma alkaline phosphatase are found. Decalcification of the bones leads to pains in the bones, deformities and fractures, and, frequently, urinary calculi. Cysts in the bones are another characteristic of this disease. It is due to a tumor of a parathyroid gland, and the treatment consists in its surgical removal. This should be done as early as possible—before the bone changes have become irreversible.

Parathyroid hormone is sometimes given as treatment in lead poisoning. In chronic lead poisoning the metal is deposited in the bones, displacing calcium from the bone salt. The hormone tends to release the lead just as it does the osseous calcium, sending it into the blood and permitting its elimination by the kidneys. However, if this occurs too rapidly, the presence of large amounts of lead in the circulation may have serious effects.

## THE PITUITARY GLAND

The pituitary or hypophysis is a small ovoid gland located at the base of the brain. It is attached to the infundibulum, the tubular stalk of the tuber cinereum, a diverticulum of the third ventricle, immediately behind the optic



chiasma, and occupies a depression of the sella turcica in the floor of the cranium. From our standpoint, the gland may be considered to consist of (1) the anterior lobe, the largest and most important part, the dominant endocrine structure, (2) the posterior lobe, which also possesses endocrine activities, and (3) the intermediate lobe, which lies between the other two and which may have hormonal functions. Methods of study of the functions of the gland begin with removal of it in animals. Because of its peculiar anatomical position, this is quite a difficult procedure, and injury to the near-by nervous structures is very likely to occur. However, in recent years the technique has been improved so that this operation is now frequently performed and much information has been gained which is applicable to human physiology. Birds, such as pigeons and ducks, have been used successfully, as have the common laboratory mammals, particularly the rat.

In the young mammal hypophysectomy results in a cessation of growth and retardation in development both physically and mentally. The animals generally remain immature, their sexual glands do not develop, the epiphyses do not unite, and the first teeth are retained. If the operation is performed upon an adult animal, there is an almost immediate effect upon all the other glands of internal secretion. The testes, ovaries, and secondary sexual organs atrophy. The thyroids, parathyroids, and adrenal cortex become smaller in size and their activity diminishes. In addition, the animal is apathetic, loses its appetite, and becomes emaciated. Protein, carbohydrate, and fat metabolism all become deranged. One evidence is the hypersensitiveness of the hypophysectomized animal to insulin, and its resistance to the hyperglycemic effect of adrenalectomy.

It is obvious that the pituitary must exert powerful influences upon many other glands of internal secretion and, either directly or indirectly, upon all physiological activities. In fact, it has been called the "master gland," since its secretions seem to control those of most of the other important endocrine glands. To be more specific, it is the *anterior* lobe of the pituitary which controls most of these vital activities. The posterior lobe should not, however, be disregarded, and for convenience it will be considered first.

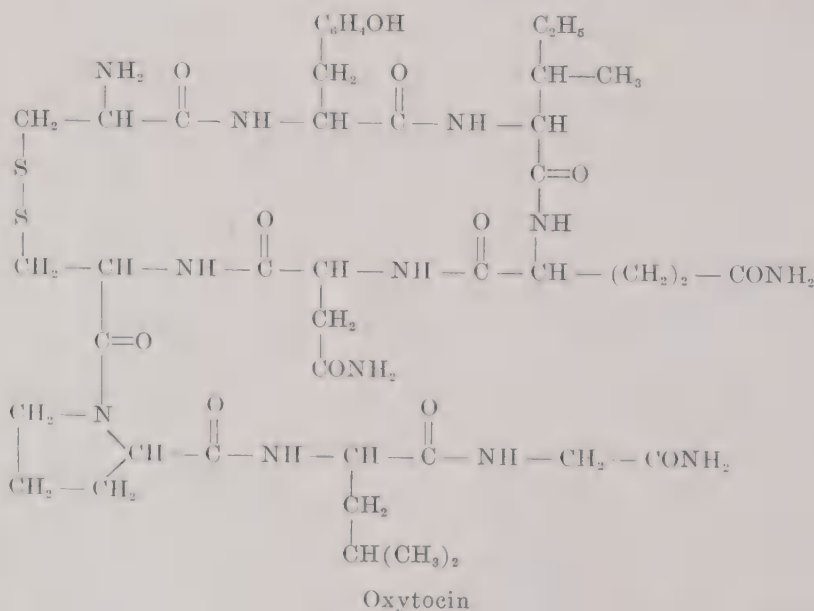
### The Posterior Pituitary Lobe

It has been practically impossible thus far to remove the posterior lobe of the pituitary without injuring the anterior lobe or other structures. The most nearly successful experiments have resulted in an increased urinary output coupled with an increased water intake. This resembles the clinical condition known as *diabetes insipidus*. As a result, the hypothesis that this disease is due to a lack of a hormone elaborated by this gland is commonly held today. In diabetes insipidus tremendous quantities of water are taken in, as much as 30 liters a day, with an excretion of almost as much urine. Most cases yield to subcutaneous administration of posterior pituitary extract. Another effective and convenient method of administration is by absorption by the nasal mucosa. Small wads of cotton soaked with the solution are inserted in the nostril, or the dry powdered extract is blown up the nostril with an insufflator. The site



of action of the hormone is believed to be the distal renal tubules, where about 10 or 15 per cent of the glomerular filtrate is actively reabsorbed. (See page 497.)

Two other effects of posterior pituitary extracts were discovered as a result of studies of their pharmacological effects upon animals and isolated tissues. The first is a marked rise of blood pressure and the second is a contraction of smooth muscle, particularly uterine muscle. Kamm has separated the extract into two fractions: one, having preponderantly pressor and antidiuretic action, termed *vasopressin* ("pitressin"), and the other, having mainly the effect upon smooth muscle, called *oxytocin* ("pitocin"). Therapeutic doses of these preparations have little effect upon blood pressure in the human being, but oxytocin finds application in obstetrics when it is necessary to stimulate uterine contractions. Another function of oxytocin is to cause milk ejection in the lactating mammary gland. This effect is probably necessary in normal lactation. Du Vigneaud and his group have purified both the oxytocic and vasopressor fractions to a very high degree. Pure oxytocin, on hydrolysis, yields one equivalent each of leucine, isoleucine, tyrosine, proline, glutamic acid, aspartic acid, glycine, and cystine and three equivalents of ammonia. It appears to be a polypeptide with a molecular weight of about 1,000, with a cyclic disulfide structure. The following structure has been assigned to it, and a substance has been synthesized on this basis, by the same investigators. This seems to be identical with natural oxytocin physically, chemically, and physiologically. It is the first synthesis of a polypeptide hormone.



Although it has been possible to separate the posterior extract into two, and perhaps even three separate fractions, there has remained the possibility that all three hormones may be linked together in one giant composite molecule. If this is true, then the fractionation has meant that this molecule was split

into three smaller molecules, each with its characteristically active grouping intact. Recently van Dyke and his collaborators have isolated a protein which seems to be pure and possesses pressor, oxytocic (i.e., stimulating the contraction of uterine muscle), and antidiuretic activity. This may mean that the posterior pituitary secretes only one hormone having this triple physiological effect.

### The Anterior Pituitary Lobe

The anterior lobe secretes a number of important hormones. All of them are believed to be proteins, and several have been isolated in pure form. Those which cause other glands to function or increase their activity are termed "trophic" hormones from the Greek *trophein*, to nourish, and this term is used as a suffix. Thus there are thyrotrophic, adrenotrophic hormones, etc. The thyrotrophic hormone has the specific effect of increasing the amount of hormone released by the thyroid gland; the adrenotrophic affects the adrenal cortex similarly, etc. There is good evidence that the rate of secretion of a trophic hormone is inversely proportional to the concentration in the blood of the hormone with which it is related. For instance, a high blood level of thyroid hormone tends to inhibit the anterior pituitary secretion of thyrotrophic hormone, and a low level causes an increased production of it. The regulatory effect of this mechanism is evident.

**Growth (Somatotrophic) Hormone.**—The presence of an anterior pituitary hormone, which influences growth, is indicated on the one hand by the fact that hypophysectomy inhibits growth and on the other by the occurrence of gigantism as a result of pituitary hyperfunction. Gigantism is not infrequent in man; the "tallest man" in the circus is a person whose pituitary was overactive during childhood, before the closure of the epiphyses limited the further growth of his long bones. Such individuals may be quite normal mentally and, except for their size, physically. (Fig. 86.) It is considered dangerous to attempt to stop the growth of these subjects by irradiating their pituitary glands because of the possible harmful effects to the other important functions of the gland.

If the overactivity of the gland occurs in an adult, that is, after the closure of the epiphyses, a condition known as acromegaly occurs. Here the bones become mishapen, particularly the bones of the face. There is also an excessive growth of fibrous tissue, resulting in thickened nose, lips, eyelids, and broadened finger tips. (Fig. 87.) Acromegaly is sometimes caused by a tumor of the anterior pituitary, and in such a case surgery may be attempted.

The opposite effect is seen in pituitary dwarfism. It is said that if no other adequate cause for retarded growth can be found, such as hypothyroidism, the trouble is likely to be hypopituitarism. (Fig. 88.) In such dwarfs the bodily proportions are relatively normal but the sex organs are underdeveloped. The administration of anterior pituitary extracts sometimes results in considerable growth of such dwarfs.

Recently Li and Evans have succeeded in isolating from the anterior lobes of ox pituitaries a protein which they consider the pituitary growth hor-



Fig. 86.—Gigantism. Height, 6 feet, 8 inches. Note the disproportionate length of the limbs. (Courtesy Dr. Robert T. Frank.)



hormone. It is a protein that can be destroyed by heat, inactivated by proteases, and precipitated by ordinary protein precipitants. It has a molecular weight of about 44,000 and behaves as a single substance in electrophoresis. This protein seems to consist of two polypeptide chains, one having alanine and the other phenylalanine as the N-terminal amino acid. It causes growth in hypo-



Fig. 87.

Fig. 87.—Acromegaly. Note overgrowth of the bones of the jaw, nose, hands, and feet. (Courtesy Dr. Thomas H. McGavack.)

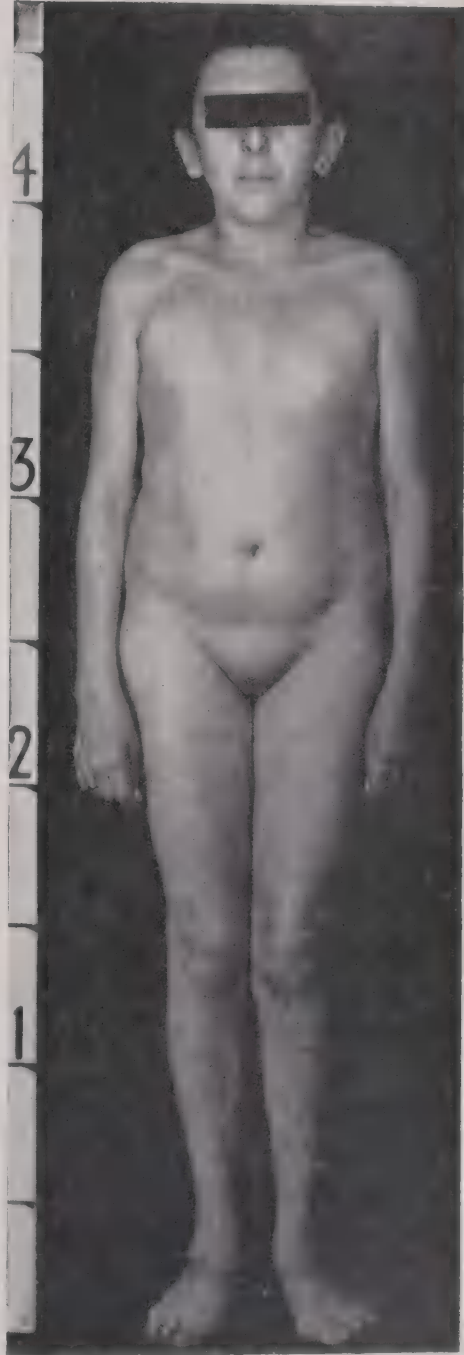


Fig. 88.

Fig. 88.—Pituitary dwarfism. Age, 22 years. Secondary sexual characters undeveloped. (Courtesy Dr. Thomas H. McGavack.)

physectomized rats and is responsible for the effects upon carbohydrate metabolism and ketogenesis, which will be discussed later. Indeed, the growth-promoting effect may be due to a depression of the oxidation of both proteins and carbohydrates, thus making these substances available for physiological needs, including growth. Since the utilization of carbohydrate and of protein is dependent to a considerable degree upon insulin, it is not surprising to note that the increase in tissue-protein anabolism, or growth, occurs as a result of the synergistic effect of these two hormones. Insulin alone cannot cause such action in normal animals, and growth hormone alone cannot do so in depancreatized animals. (Lukens.) Young's view is that the growth-promoting action of the anterior pituitary hormone depends on the availability of extra insulin, secreted by the islands of Langerhans *under its influence*.

The interrelationship of several glands of internal secretion to pancreatic diabetes was discussed in Chapter 16. The particular influence of the pituitary may, however, be emphasized at this time.

It will be remembered that the removal of the pancreas produces hyperglycemia and the extirpation of the hypophysis results in hypoglycemia. The two glands seem to have opposing effects. This was shown very strikingly by Houssay in 1930. He found that the removal of the pituitary gland lessened the severity of the diabetes produced by depancreatization. The animal is not cured of diabetes by any means. The hyperglycemia, however, is not as severe as in the animal deprived only of its pancreas. Moreover, the survival of the "Houssay" animal is much longer than the simple diabetic animal, if neither receives hormone therapy. The Houssay animal is more sensitive to the injection of insulin or anterior pituitary extract. That is, insulin produces more profound hypoglycemia and pituitary extract more readily causes hyperglycemia in such animals, apparently because the antagonizing factor is absent in each case. This demonstrates very beautifully the interplay of hormones. Seldom does one hormone have an isolated or unmodified effect. Usually it not only causes its own pharmacological or nutritive action, but also either stimulates or inhibits the secretion of other hormones, or modifies the effect of some other hormone.

In man the relationship between the two glands is observed in acromegaly. In this condition of hyperactivity of the pituitary gland, there is frequently seen hyperglycemia and glycosuria. These effects of the anterior pituitary are probably explained on the basis of the action of ACTH, since this causes the adrenal cortex to secrete glucocorticoids.

Injection of anterior pituitary extracts has variable effects upon the normal animal. In some cases a rise in blood sugar is seen and in some, a fall. This seems to depend upon whether the animal is fasted or is well fed, upon the species of animal employed, upon the number and amount of the doses given, and upon other factors. Therefore, it is not surprising that the existence of a number of different principles was formerly postulated. These were called the pancreatotrophic, glycostatic, and diabetogenic hormones. It is now rather definite that all of these effects can be ascribed to the growth hormone.

Thus, in addition to the influence of the adrenocorticotrophic hormone, which stimulates the production of the adrenocortical steroids, and thus indirectly affects carbohydrate metabolism, the anterior pituitary has a direct effect. It has a marked anti-insulin action by inhibiting the peripheral utilization of sugar. (De Bodo and Sinkoff.) On the other hand, a single injection administered to fasting rats produces a moderate hypoglycemia. (Milman and Russell.) These contradictory effects may be related to the fact that, while the growth hormone stimulates the secretion of the extra insulin, it also stimulates the secretion of glucagon and diminishes the sensitivity of tissues to insulin. Apparently the type of effect produced by the growth hormone will depend upon the balance of these two activities and upon other factors. Usually in young animals the growth effect prevails, while in adult animals, especially carnivores, the diabetogenic action may be seen. (Young.)

The injection of anterior pituitary extracts also causes an increase in the formation of ketone bodies. For a time this was believed to be a distinct hormone, a *ketogenic factor*, but is now believed to be still another action of the growth hormone. This type of effect is best shown by injection of the growth hormone into starving animals or those on a high fat diet; i.e., those animals having some degree of ketosis already. It is natural to assume that this action is an intensification of the normal production of ketone bodies from fatty acids by the liver, but there is no definite proof of the mechanism of this ketosis as yet.

**Thyrotrophic Factor.**—It was stated previously that removal of the hypophysis results in a decrease of the size of the thyroid gland. This can be prevented by the injection of certain extracts of the anterior pituitary, and if such extracts are injected into normal animals, the thyroid will hypertrophy. The active principle involved is called the thyrotrophic factor, or hormone. It has been obtained in a high degree of purity by Ciereszko and White. It is a protein of relatively low molecular weight.

This hormone's action is upon the thyroid, stimulating it to secrete the thyroid hormone. Thus it has an indirect action upon general metabolism. The removal of the anterior pituitary, or the inhibition of its secretory activity, has as a result equivalent to hypothyroidism. The injection of the hormone, on the other hand, is similar to the administration of thyroid, or to the production of hyperthyroidism with the following effects: (1) enlargement of the thyroid gland, (2) rise in the basal metabolic rate, (3) reduction in the iodine content of the thyroid gland, (4) increase in the iodine content of the blood, (5) greater rapidity of the heart rate, and (6) exophthalmus. There are other results besides these, but it is possible that they are due to the presence of other factors.

The disorders of the thyroid, observed clinically, may thus be due either to a primary thyroid disease or to an effect upon the thyroid, secondary to some disorder of the anterior pituitary. It should also be mentioned in this connection that these two glands seem to have a reciprocal relationship; that is, after thyroidectomy the pituitary glands become enlarged.

**Adrenocorticotrophic Factor.**—One of the most important principles elaborated by this gland is the adrenocorticotrophic hormone, or ACTH. This



“trophic” substance causes the adrenal cortex to secrete its steroids more actively. The anterior pituitary is sensitive to the requirements of the body for adrenal cortical hormones and secretes ACTH in increased quantities during stress. The ACTH then acts upon the adrenal cortex, leading to an increased output of the adrenal cortical hormones and causing hyperplasia to ensue. There is considerable evidence that this action is due to an acceleration of the *synthesis* of the corticosteroids rather than merely increasing the rate of their release from the adrenal cortex. (Haynes.) This factor, in a manner analogous to that of the thyrotrophic factor, can prevent the regression of the adrenal cortex which occurs when the hypophysis is removed. Evidence of the close relationship between these two glands is seen in the fact that in acromegaly, in which the pituitary is overactive, the adrenal cortex also shows an increase in size. The adrenocorticotrophic factor is a protein and has been isolated in a highly purified state by workers in two laboratories, working independently and using pituitaries of different species. (Li; Sayers.) It has a molecular weight of about 22,600 and can be heated to 100° C. for a brief period without loss of activity. When injected into animals this substance, of course, produces all the varied effects which are caused by the adrenal cortical hormones themselves. Furthermore, it can evoke these effects in hypophysectomized animals.

The influence of the adrenocorticotrophic factor, or indeed of any factor, upon the adrenal cortex can be followed by determining either the cholesterol or the ascorbic acid content of the cortex. From such studies, it has been shown that prior treatment with cortical hormone prevents the usual fall in ascorbic acid when the animal is exposed to cold, trauma, etc.; that is, the blood level of cortical hormone seems to influence the rate of secretion of adrenocorticotrophic hormone. Other factors also seem to be concerned in the regulation of the secretion of ACTH by the anterior pituitary. The hypothalamus may have some action, either by a nervous mechanism, or by some secretion. Finally, there is some evidence that epinephrine, or to a less degree, norepinephrine, may stimulate the release of ACTH, especially during acute stress, which activates the sympathetic nervous system.

The employment of ACTH for therapeutic purposes is discussed on page 679.

**Lactogenic Hormone.**—The demonstration of a factor of the anterior pituitary stimulating the secretion of milk was accomplished by Riddle, who called the hormone “*prolactin*.” It can initiate lactation not only in the mature female breast, but also in the breast of an immature female or of a male if they have been properly prepared by a preliminary treatment with certain other hormones.

Prolactin was the first anterior pituitary hormone to be obtained in pure form. It is a protein and has been crystallized by White, Catchpole, and Long (Fig. 89). In this pure form it has no growth-promoting, thyrotrophic, diabetogenic, adrenotrophic, or gonadotrophic activities. It is thermolabile and is destroyed by tryptic digestion. Clinically, prolactin has been used in delayed or insufficient lactation with encouraging results. (Kurzkrok.)

Prolactin alone has little or no influence upon the growth or development of the undeveloped mammary gland, although it may have some effect upon the growth of the gland in association with the hypertrophy coincident with the induction of lactation. The lactogenic hormone initiates lactation in mammary glands suitably prepared by gonadal hormones. The estrogenic hormones do not induce mammary growth in hypophysectomized animals. Growth does occur, however, in such animals if they receive lactogenic hormone in addition. Prolactin also has gonadotrophic properties, in the rat at least, in that it maintains functional corpora lutea in hypophysectomized animals.

**Gonadotrophic Factors.**—It has been mentioned that hypophysectomy results in atrophy of the primary and secondary sexual glands. These may be restored to a normal function by administration of extracts of the anterior lobe or by implantation of living anterior lobe tissue. Since it makes no difference whether the pituitary glands are derived from males or females, it is evident that the pituitary hormones involved are identical, and the type of effect produced is determined by the sex of the animal affected.



Fig. 89.—Crystalline prolactin ( $\times 900$ ). (From White, A., Bonsnes, R. W., and Long, C. N. H.: J. Biol. Chem. **143**: 447, 1942.)

Three anterior pituitary hormones are now recognized as “*gonadotrophins*,” that is, hormones which stimulate the sex glands, all of which are active in the female and only two in the male. They are the follicle-stimulating hormone (F.S.H.), the luteinizing hormone, or interstitial cell-stimulating hormone (I.C.S.H.), and prolactin. The interrelationships of the gonadotrophic and the sex hormones are exceedingly complicated. The simplified account which follows, although based on the present state of knowledge, is quite likely to require modification in the future as new facts are discovered. (See Fig. 90.)

In the female, F.S.H. stimulates the growth of a graafian follicle in the ovary in preparation for the release of the ovum. During this phase of the

menstrual cycle the follicle itself secretes a "female sex hormone," an estrogen called "*estradiol*." The estradiol induces a proliferation and thickening of the endometrium and an increase in its vascularity, thus preparing the uterine wall for the reception of the fertilized ovum. The luteinizing hormone controls the development of the corpus luteum. The corpus luteum secretes another sex hormone, "*progesterone*," while the secretion of estrogen is gradually diminishing. Prolactin now maintains the corpus luteum in an active state for the continued production of progesterone, and progesterone is one of the hormones necessary for maintaining pregnancy.

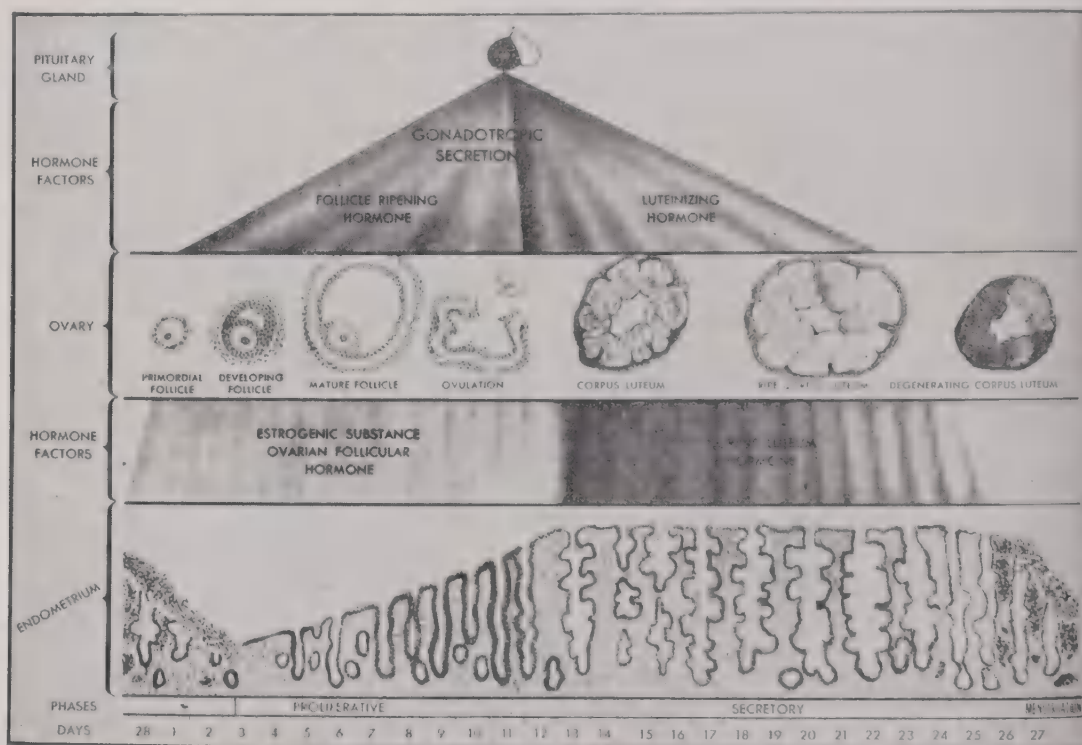


Fig. 90.—Pituitary control of the sexual cycle of the female. This is a simplified diagram and does not indicate all interrelationships; e.g., follicle ripening hormone requires the synergistic action of the luteinizing hormone for the production of estrogenic substance, and the luteinizing hormone is complementary to the action of prolactin. (Courtesy The Armour Laboratories, Chicago, Ill.; drawn by F. Netter, M.D.)

In the male, F.S.H. induces the growth of the testes by causing proliferation of the sperm-forming tissue. Thus mature spermatazoa are produced. The secretion of "*testosterone*," the "male sex hormone," is stimulated by I.C.S.H. The function of testosterone is to sustain spermatogenesis and to develop the secondary or accessory sex organs, the vas deferens, prostate gland, and the vesicles.

Both F.S.H. and I.C.S.H. have been isolated after much difficulty. They are glycoproteins, which are soluble in water, destroyed by tryptic digestion, by dilute acids and bases, and by heating above 50° C. The follicle-stimulating hormone has a molecular weight of 67,000 and an isoelectric point of pH 4.5. In contrast to trypsin digestion, peptic digestion at pH 4.0 results in a product which retains the activity of the native protein.



### Chorionic Gonadotrophin

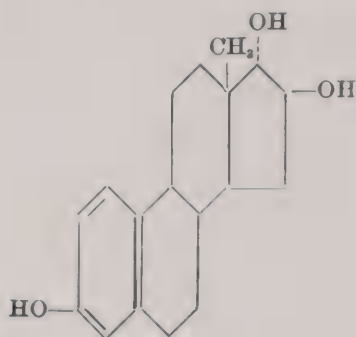
Chorionic gonadotrophin was discovered by Aschheim and Zondek in the urine of pregnant women and became the basis for their pregnancy test and modifications of it. This hormone is produced by the placenta and appears in the urine shortly after pregnancy begins. It also is formed whenever there is abnormal chorionic proliferation, such as hydatidiform mole or chorionepithelioma. Such cases must be ruled out when making the test. The injection of urine containing this gonadotrophin into immature female white mice under standard conditions causes hemorrhage of an ovarian follicle in about ninety-six hours. The Friedman modification uses a mature unmated female rabbit. In such an animal the injection of the hormone results, within twenty-four to forty-eight hours, in follicular rupture and corpus luteum formation. The results in both species can be observed macroscopically. This gonadotrophin is now considered to possess more luteinizing than follicle-stimulating power. It is a glycoprotein containing galactose and has been purified to a high degree. (Gurin.) It is usually called the "anterior pituitary-like" hormone, or A.P.L., but this is not an accurate term because it is not effective in hypophysectomized rats, whereas the pituitary hormones are.

### OVARIAN HORMONES

During the growth of the follicle under the influence of F.S.H., estrogen is secreted, as has been seen. Estrogen is a generic term for a substance which induces estrus. This is a cyclic phenomenon of the female reproductive system. The stages and timing differ in various species, but in general there is first a proestrus period during which the follicle ripens and the organs of reproduction develop. This is followed by estrus, the period of "heat," in which the female will receive the male. Ovulation takes place toward the end of estrus, either spontaneously, or, as in the rabbit, after mating. Then follow a period of retrogression of the accessory reproductive organs and a period of sexual inactivity. In some animals, such as the mouse or rat, the estrus cycle is accompanied by characteristic changes in the vaginal epithelium. Vaginal smears, when viewed microscopically, reveal whether estrus is occurring or not, and this technique is used to determine qualitatively and quantitatively the estrogenic activity of hormones of synthetic substitutes.

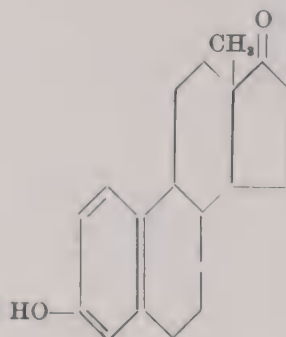
In the human being the reproductive phenomena also show a periodicity, but this is not the same as estrus. The follicle matures and the ovum is discharged, not during menstruation, but at the mid-interval of the cycle, whereas during estrus in other animals the vaginal bleeding occurs at a time near ovulation. However, the estrogen in the human subject has a definite effect upon the female organs, inducing growth of the vagina, uterus, mammary glands, and accentuating secondary sexual characteristics. Crude extracts of ovary or follicular fluid will produce estrus experimentally in immature rats. This was shown by Allen and Doisy, and led to the isolation, crystallization, and identification of "*theelin*." This was the name originally given to the first estrogenic substance obtained by Doisy, but now the term "*estrone*" is

commonly used. There are known to be several naturally occurring estrogenic compounds. The most important ones are (1) estrone or theelin, (2) estriol or theelol, (3) estradiol or dihydrotheelin, (4) equilin, and (5) equilenin. These and several other natural estrogens are steroids. Their formulas are:



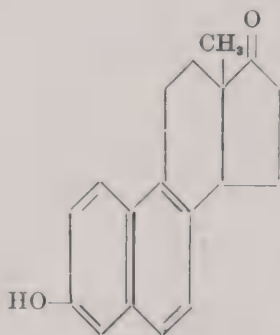
Estriol

( $\Delta^{1,3,5:10}$ -Estratriene-3,16,17[ $\alpha$ ]-triol)



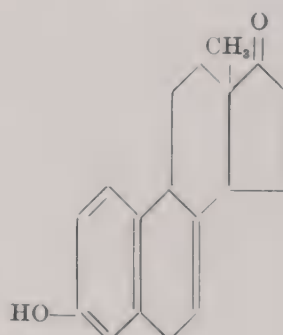
Estrone

( $\Delta^{1,3,5:10}$ -Estratriene-3-ol-17-one)



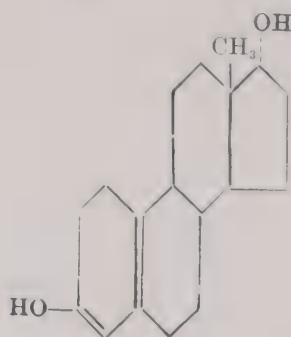
Equilenin

( $\Delta^{1,3,5:10,6,8}$ -Estrapentaene-3-ol-17-one)



Equilin

( $\Delta^{1,3,5:10,7}$ -Estratetraene-3-ol-17-one)

 $\alpha$ -Estradiol

( $\Delta^{1,3,5:10}$ -Estratriene-3,17[ $\alpha$ ]-diol)

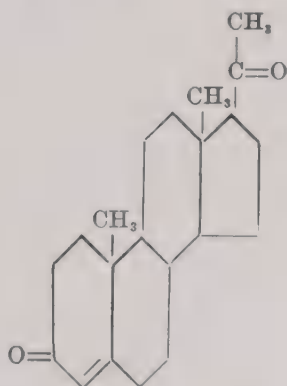
Although estrone was the first of these to be isolated, and for a long time was called the female sex hormone, it is not as powerful as estradiol. In fact, there is a tendency to regard estradiol as the true follicular or ovarian hormone. It is believed that as estradiol circulates it is converted by some organ to estrone, and this to estriol. The sequence of reactions is probably: estrone  $\rightarrow$  16-ketoestrone  $\rightarrow$  16-keto- $\alpha$ -estriol  $\rightarrow$  estriol. (Stimmel.) Estriol is then united with glucuronic acid, forming estriol glucuronide which has little

or no physiological action. Just how much of the hormone is metabolized and excreted in this way is not known. When estradiol is administered to laboratory animals, less than 20 per cent is excreted, partly in this conjugated form; the remaining 80 per cent is destroyed in the body. The conjugation may be a step preliminary to its utilization by tissues or to its ultimate destruction. The liver is the site of the destruction of estrogens in some experimental animals and also probably in the human subject. The ability of the liver to metabolize these hormones is dependent upon nutritional factors, among which is the intake of protein. (Jailer and Seaman.)

### Corpus Luteum Hormone

The corpus luteum produces progesterone. This hormone seems to be responsible for continuing the development of the uterus initiated by estradiol and converting the endometrium to a secretory stage. Indeed it has very little effect unless the uterine development has been started by the estrogenic hormone. Progesterone has a number of other effects, all of which have some bearing upon the reproductive cycle. It inhibits ovulation and has an influence upon the growth of the mammary glands, producing development of the acinar structures. The absence of this hormone, brought about by removal of the corpus luteum during early pregnancy, causes a failure in nidation and implantation of the ovum, or expulsion of the ovum or embryo if it is already implanted.

Although there may be several progestational hormones, the most active one is progesterone, which has been isolated and has the following formula:



Progesterone  
( $\Delta^4$ -Pregnene-3,20-dione)

Note the close resemblance to the structure of 11-desoxycortisterone (page 569). It is not surprising, therefore, to find that progesterone has certain adrenocortical properties; namely, those influencing salt and water. Progesterone is soluble in most organic solvents and in the vegetable oils but is insoluble in water. It is used clinically to some extent, particularly in the treatment of menorrhoea, i.e., absence of menstruation in young women.

**The Menstrual Cycle.**—At this point it may be of value to outline the effects of the various hormones on the menstrual cycle. True menstruation



occurs only in man and in the members of a closely related group of the primates. Under the influence of the pituitary gonadotrophic hormone, thylenin, the follicle matures and an increasing amount of estradiol is formed. This occurs during the first two weeks of the cycle. Under the influence of estradiol, the endometrium increases in thickness and vascularity up to the time of ovulation. The follicle then ruptures and liberates a mature ovum, after which the luteinizing hormone causes the ruptured follicle to change to a corpus luteum, which forms progesterone. Progesterone, in turn, causes the endometrium to assume a turgid secretory condition and to be ready to receive and maintain a fertilized ovum. If the ovum is not fertilized, the corpus luteum regresses, progesterone diminishes in amount, and the endometrium breaks down and menstrual bleeding occurs. If the ovum is fertilized, the secretion of progesterone continues and is necessary for the maintenance of pregnancy. Prolactin, it will be remembered, aids in continuing the secretion of progesterone. (See Fig. 90.)

**Other Effects of Estradiol.**—Besides its action on the endometrium, estradiol seems to maintain the normal size and function of the various parts of the female reproductive organs. It also promotes the growth of the duct tissue of the breasts. Moreover, it appears to have a controlling action on the secretion of the anterior pituitary, so that the ovary and pituitary seem to have reciprocal effects. Thus, indirectly, the ovarian hormone may have an influence upon the other endocrine glands through its action upon the pituitary. A number of other effects have been shown to be due to estradiol, including the stimulation of the growth of certain epithelial tissues, especially the mucosa of the vagina and of the nose.

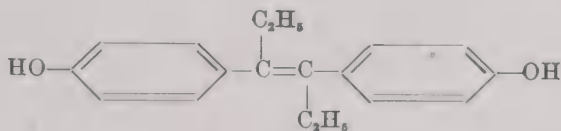
**Therapeutic Uses of Estradiol.**—In common with the other sex hormones, and indeed most of the hormones of whatever origin, estradiol has been recommended for a multitude of therapeutic purposes, often with no justification. It is a very potent substance and may have harmful effects if used indiscriminately. Fortunately, upon cessation of administration, the unpleasant symptoms tend to disappear. If employed on the basis of its known physiological effects, it becomes a very useful agent to the physician.

It is said to be effective in developing the female sexual organs in sexual infantilism. In juvenile vaginitis due to gonorrheal infection and in other forms of vaginitis it is given in order to stimulate proliferation of the mucosa. It promotes the growth of the breasts under certain conditions, but also is said to inhibit the pituitary in the control of excessive milk secretion.

This inhibiting effect upon the pituitary is also believed to be the basis for the use of estrogens in controlling abnormal symptoms of the menopause. The menopause is that period in which menstruation ceases and the woman is no longer capable of becoming pregnant. In most cases it occurs without particular disturbance, but in about 15 per cent there ensue a number of distressing symptoms. These are chiefly cardiovascular, vasomotor, and nervous in nature. To some extent they may be controlled by estrogenic substances. Since there is no marked decrease in the estrogenic content of the

blood in these cases, the beneficial action of the estrogens is attributed, as mentioned previously, to their inhibiting action on the pituitary.

**Stilbestrol.**—Stilbestrol is a synthetic product which has marked estrogenic properties. As can be seen from its formula, it does not resemble the steroids from a chemical standpoint. However, it produces practically all the physiological effects that estradiol does. It is administered by mouth. In some cases unpleasant "side effects" are seen, but usually if the dosage is carefully regulated these do not occur. The formula of the more potent diethyl derivative is:

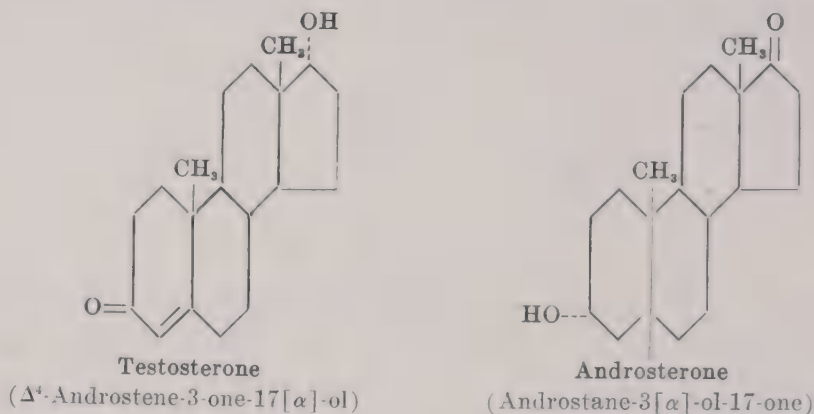


Diethylstilbestrol

### THE TESTICULAR HORMONE

One of the first attempts to demonstrate the presence of a male sex hormone was that of Brown-Séquard. In 1889, at the age of 72, he gave testicular extracts to himself and felt that he experienced increased vigor and strength. These and similar experiments have not been convincing, and there is no evidence that the testicular secretion has any relation to the phenomenon of aging. The testes, however, do secrete a definite hormone. As has been seen, the actual production of this secretion is regulated by the anterior pituitary. It can be demonstrated in several test animals. For example, injection of testicular extracts into capons causes the growth of the comb, wattles, and ear lobes (Gallagher and Koch). It has a similar growth-promoting effect upon the combs of male chicks. It also inhibits ovulation in hens and has the curious effect of causing the ovipositor of the female bitterling, a small fish, to increase in length, a phenomenon which occurs during the natural sexual cycle. The effective substance, or substances, can be isolated not only from testicular tissue, but also from urine. The androgens, as they are generically called, also have some estrogenic properties. Thus the term male hormones is not strictly applicable. Furthermore, the occurrence of estrogenic and androgenic substances is seen in the urine of both sexes, although androgens usually predominate in the urine of males and estrogens in that of females. The chief functions are to produce normal development of the male reproductive organs and to maintain the secondary male characteristics. Under its influence the descent of the testes occurs. Testosterone is also effective in sustaining spermatogenesis (Smith). The secondary male characteristics which develop in its presence are the deep voice, the growth and pattern of facial and body hair, and the male type of skeletal muscular development. It inhibits mammary development and function and stimulates libido in both the male and the female. The male hormones constitute one factor in the production of baldness. Age and inheritance are other factors involved in bringing about this condition, but baldness does not ensue without androgenic stimulation. (Hamilton.)

The structure and the chemistry of the androgens have been worked out largely by Butenandt and by Ruzicka and their colleagues. They also are steroids. Testosterone is probably the characteristic hormone, and androsterone seems to be a transformation product of it. Intermediate products are  $\Delta^4$ -androstene-3,17-dione and epitestosterone ( $\Delta^4$ -androstene-3-one-17[ $\beta$ ]-ol). (Kochakian.) The liver is probably the site of the inactivation of androgens as well as of estrogens.



Clinically, testosterone is used where the testes are absent or are unable to function. Such individuals may have an effeminate appearance and build, with broad hips and prominent breasts, feminine pattern of pubic hair, slight growth of facial hair, and a high-pitched voice. They usually are easily fatigued and have a low basal metabolic rate. These symptoms will vary greatly with the age of onset of the disorder. The administration of suitable doses of testosterone may relieve many of these symptoms. It often brings about enlargement of the sexual organs, prostatic secretion, the growth of pubic and axillary hair as well as a beard, and deepening of the voice. Such individuals acquire sexual desire and may become fathers if there is potentially functional testicular tissue present. The use of the gonadotrophic hormones is theoretically sounder if there is testicular tissue which can be stimulated. The administration of large doses of testosterone to women may have masculinizing effects.

In cryptorchidism, that is, failure of the testes to descend in the normal manner, testosterone seems to be of considerable value. If testosterone is administered in high therapeutic dosage for long periods of time (for example, 25 mg. daily for four to six weeks), atrophy of the sperm is likely to result. However, such an effect may be reversed if treatment is discontinued for a similar length of time.

**Steroid Interrelationships.**—The reader must have been impressed by the curious fact that all the sex hormones are closely related chemically. Only slight changes in certain parts of the molecule are needed to transform a “female” to a “male” hormone. It is also interesting to note that the adrenal cortical hormones are also steroids and that abnormalities in sexual development are often traced to the adrenal cortex. Furthermore, the adrenal cortex can manufacture both androgens and estrogens, and conversely, the sex hor-



ones also have some adrenal-cortical functions. Progesterone, for example, has some effect in ameliorating the symptoms resulting from adrenalectomy.

The interaction of the glands of internal secretion has been noted above several connections. Undoubtedly many other relationships obtain. Selye has shown some of these in his hypothesis of the "General Adaptation Syndrome." This states that the prolonged exposure of an individual to stress, such as pain, fear, cold, or infection, leads to (1) the alarm reaction, (2) the stage of resistance, and (3) exhaustion. Many nervous impulses come into play and are related to hormonal releases, especially those of the pituitary-adrenal system. However, from the purely biochemical standpoint, there is believed to be, during the first stage, a breakdown of body proteins with an increase in the content of protein catabolites and proteolytic enzymes in the blood. This seems to be due to a generalized disintegration of body cells. At the same time these stresses result in an enlargement of the adrenal cortex.

The hormonal defense mechanism is characterized by a *decreased* secretion of most of the anterior pituitary hormones but an *increased* output of ACTH. This causes the adrenal cortex to secrete its hormones, especially the glucocorticoids, in increased amounts. The effect of these products is manifold. There is involution of the thymus gland and other lymphatic organs. The blood count is greatly changed. Gluconeogenesis is stimulated, and a rise in blood sugar ensues. There are many other effects, and Selye considers it possible that a number of diseases may be listed as "diseases of adaptation." Among them are hypertensive diseases, nephrosclerosis, nephritis, peritonitis nodosa, rheumatic diseases, gastrointestinal ulcers, gout, diabetes mellitus, and Cushing's syndrome.

The determination of the metabolic products of some of these steroids in the urine has become of considerable clinical importance. The "17-ketosteroids" of particular are of interest. This term refers to those steroids possessing a ketone group at position 17. An example is androsterone, the formula of which is given on page 636. Other important 17-ketosteroids are two isomers of androsterone, dehydroisoandrosterone and estrone. The determination involves hydrolysis to free the steroids from their ester combinations, extraction with organic solvents, and removal of phenolic substances, including estrone. After further purification they can be determined quantitatively by color reactions. Since estrone has been removed, the figure obtained is an index of the steroid secretory activity of the adrenal glands in the female and of the adrenal glands and testes in the male.

In normal adults between the ages of twenty and forty years, the twenty-four hour excretion of the neutral 17-ketosteroids ordinarily ranges from 7 to 12 mg. in women and from 12 to 17 mg. in men. The greater output by men is ascribed to the fraction produced by the testes. The values are expressed in terms of milligrams of androsterone. In childhood the amounts rise from 1-2 mg. for children at 3 or 4 years of age to about 8-9 mg. at 11 years. Above 12 years, boys excrete 8-13 mg., and adult values are reached at the age of 18 years or even younger.

In old age, low values are found in both sexes. Pathologically, derangements of the adrenal cortex, testis, and anterior pituitary are accompanied by changes in the 17-ketosteroid output. Decreased amounts are excreted in hypopituitarism and also in acromegaly. In Addison's disease there is also a low elimination. In hypogonadism in both sexes a low output is sometimes, but not always, seen. High values are found in adrenal cortical hyperfunction or in testicular hyperfunction, as in certain tumors of these glands. However, the adrenogenital syndrome does not always result in an increase in these steroids—if it does not, it may indicate a simple hyperplasia rather than a tumor.

Recently it has been indicated that the determination of 17-hydroxycorticoid excretion in the urine affords a more sensitive measure of adrenal activity than the alteration of the 17-ketosteroid excretion. (Thorn.) The 17 hydroxycorticoids, which have been isolated from human urine, include 17-hydroxy-11-dehydrocorticosterone (cortisone), its dihydro and tetrahydro metabolic reduction products, 17-hydroxycorticosterone, estradiol, and estriol. (Schneider.)

**BIOSYNTHESIS OF STEROID HORMONES.**—By perfusing the respective glands with solutions containing labeled acetate, it has been shown that the complex steroid hormones are synthesized from the simple two-carbon chain. Thus adrenal hormones are produced by the adrenal gland, testosterone and  $\Delta^4$ -androstene-3,17-dione by the testis, and estrone,  $\beta$ -estradiol, and cholesterol by the ovary. (Werthessen.)

The relationship of the steroids to cancer is also of growing importance. There is some experimental evidence that continued overdosage with estradiol will produce tumors but that discontinuous treatments are without this effect. Progesterone and testosterone have an "antitumorigenic" action upon these tumors produced by estradiol. (Lipschutz.) Some clinical improvement is seen in some cases of metastatic carcinoma of the prostate when estrogens are administered. Castration, that is, deprivation of androgens, has a similar effect. It is also noteworthy that two carcinogenic compounds, which are not steroids, are also estrogenic. (Cook and Dodds.)

**ANTIHORMONES AND HORMONE ANTAGONISTS.**—Antihormones are substances antagonistic to their respective hormones and may be produced when the hormone is administered in large amounts and over a very long period. Collip and Anderson found that an experimental animal could be made resistant to the thyrotrophic hormone of the pituitary in this way. Only relatively few hormones have this action, and all are protein in nature. They are apparently analogous to antibodies and do not have the importance which was at first attributed to them.

Another type of antagonist may arise from the synthesis of substances having structures which differ slightly from the effective natural product. Woolley has produced compounds which antagonize thyroxine in its effect upon the tadpole. Several ethers of N-acetyl diiodotyrosine were found to protect these animals against the lethal action of thyroxine. This is an example of the biochemical antagonists which will be further discussed in Chapter 24.

## References

*General*

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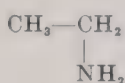
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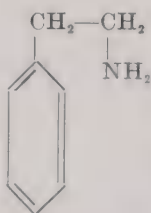
## Chapter 24

### CHEMICAL STRUCTURE IN RELATION TO BIOLOGICAL PHENOMENA

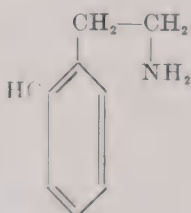
The great number and variety of constitutional effects produced by chemical compounds must indicate to any observer that physiological functions are dependent upon chemical structure. An example of a physiologically active substance, the chemical structure of which is exactly known, is epinephrine. It has a variety of effects and is known as a sympathomimetic substance; that is, its actions are similar to those produced by stimulation of the sympathetic nerves. Barger and Dale have made a study of the sympathomimetic action of compounds possessing various portions of its structure, starting from simple amines



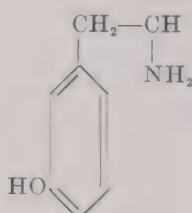
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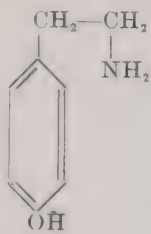
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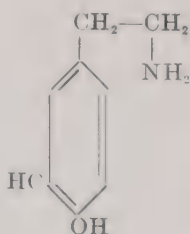
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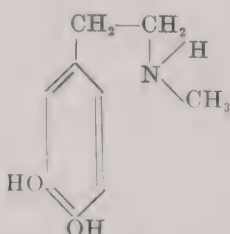
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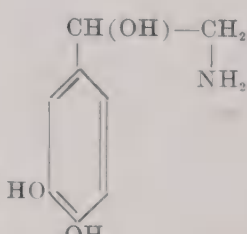
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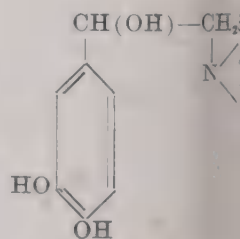
VI



VII



VIII



Epinephrine

and continuing with more and more complex compounds, until substances very closely resembling epinephrine were tested. Ethylamine (I) has no sympathomimetic properties, although some of its higher homologs have. The introduction of a phenyl group (II), however, produces an active compound, but the introduction of a methyl group into the  $\text{NH}_2$  of II or the attachment of an hydroxyl group to its  $\alpha$ -carbon (analogous to those substitutions in epinephrine) does not increase the activity of this compound. An hydroxyl in the ortho position (III) had no influence on the activity of II, but an hydroxyl in either the meta (IV) or para (V) position increased the activity several times. Here again the introduction of a methyl or hydroxyl group had no appreciable ef-



ct. Two hydroxyls, in the meta and para positions (VI), and only in those two positions, enhanced the activity considerably, but these still are not as powerful as epinephrine, even with a methyl group introduced into the amine (VII). However, the attachment of a hydroxyl to the alpha carbon (VIII) produces a compound slightly more potent than epinephrine itself. This is norepinephrine. (See page 604.) It is thus seen that the structural components and the manner of arrangement of these components contribute to the functional activity of this hormone. The study of this type of relationship belongs to the realm of pharmacology and this emphasizes again how the various fundamental sciences overlap.

Immunology is another sister science which is now being drawn closer to biochemistry. Immunologically active substances would also seem to offer material for a study of the relationship of chemical structure to biological function. They induce reactions which in many instances are highly specific, indicating a production of substances with peculiarly definite configurations or molecular rearrangement of pre-existing compounds. Antigens and antibodies are conspicuous examples. Antigens are substances which stimulate the formation of antibodies and possess the power of combining with their homologous antibodies. It was formerly thought that all were of a protein nature, and, indeed, every protein can act as an antibody. However, other types of compounds are antigenic, or perhaps take part in antigenic reactions. Notable examples are the specific polysaccharides which have been isolated from the different types of pneumococci (Heidelberger and Avery). These have been highly purified and have been used in immunizing men against pneumococcal pneumonia with a high degree of success (MacLeod). Dextrans also have been shown to have immunological properties. Most antibodies formed are in the  $\gamma$ -globulin fraction but some are  $\beta$ -globulins. Among the former are the antibodies to mumps, influenza, and most virus proteins. The diphtheria antibody may be a  $\beta$ -globulin. Probably these specific properties are due to definite changes in the configurations of the molecular structure of these proteins brought about in some way by the action of the antigens. However, the inner structure of the antigens and the antibodies is unknown, and the relationship of structure to function remains to be elucidated.

The relationship of chemical structure to biological activity has been brought out also in studies along several other lines. Two important topics may be considered under the headings Detoxication and Biochemical Antagonism.

### DETOXICATION

It was stated in Chapter 11 that the quantities of toxic products absorbed from the large intestine are not very great, and even these small amounts are detoxicated. By detoxication is meant all the processes whereby noxious substances are rendered less harmful or more easily excreted. This desirable result is effected by mechanisms which the body ordinarily uses in its normal metabolic processes. That is, detoxication mechanisms are probably not specific in regard to toxic substances absorbed from the bowel, nor indeed for any substances

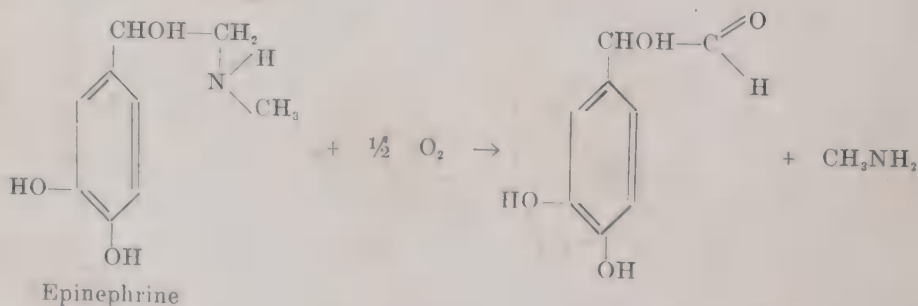
simply because of their toxicity, but rather they are directed toward particular types of substances which arise in cellular activities. Some of these substances happen to be harmful and are rendered less harmful by the chemical transformations resulting from the usual activities of enzymes upon definite chemical groups or linkages. Hence it is not surprising to note, on the one hand, that these detoxication systems operate against poisonous substances of whatever origin or however introduced. On the other hand, the operation of some of these "detoxicating" systems does not necessarily imply or guarantee that a nontoxic or even a less toxic substance will be produced.

The detoxication mechanisms fall into the following categories: oxidation, reduction, conjugation, and hydrolysis. Of these, perhaps the most important are oxidation and conjugation. Quick has presented considerable evidence to show that a very important factor in detoxication is the conversion of a weakly acidic substance to a strongly acidic one. The kidney apparently can excrete stronger acids and their salts more readily than weaker ones. The processes mentioned generally bring about this result.

**Oxidation.**—Oxidation usually occurs first and sometimes is followed by conjugation. Indole is an example (see page 521). It is first oxidized to indoxyl, which is then conjugated with sulfuric acid. Some substances can be completely decomposed by oxidation. Ethyl alcohol, in moderate amounts, can be burned by the body to carbon dioxide and water. The fact that methyl alcohol yields intermediate toxic products, formaldehyde and formic acid, in the same sort of combustion, emphasizes the fact that these reactions are general metabolic mechanisms, which may fall short of actual detoxication.

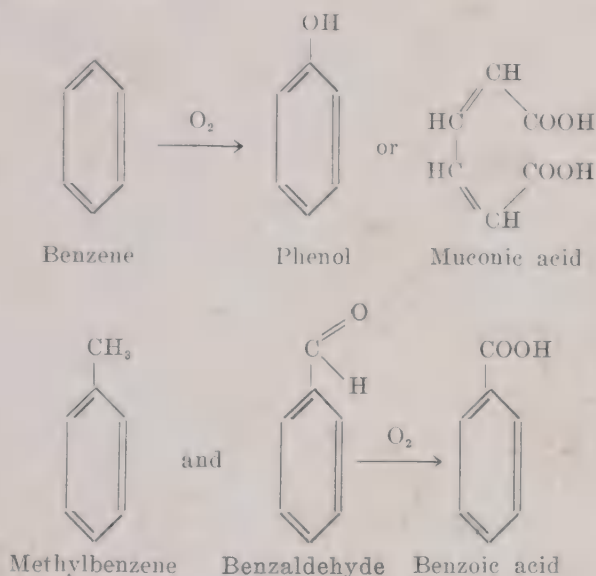
Aliphatic amines are completely oxidized by the body. An enzyme, an amine oxidase which accomplishes this, has been found to occur in brain and other tissues. (Pugh and Quastel.) In the case of butyl amine, a product of the reaction is acetoacetic acid, which is, of course, a normal metabolite. Blaschko and co-workers have shown that there is present in liver, intestine, and other tissues a similar enzyme which catalyzes the oxidative deamination of epinephrine and related amines.

The reaction is as follows:



It was pointed out on page 455 that phenyl-substituted fatty acids are oxidized by beta oxidation, losing two carbons at a time in the process. The final products are phenylacetic acid, if the chain contains an even number of carbons, and benzoic acid, for those having an odd number of carbons. Both

phenylacetic acid and benzoic acid are then conjugated with glycine to yield phenylaceturic acid and hippuric acid, respectively. In man, however, phenylacetic acid is conjugated with glutamine, as will be shown later. Benzene itself is slowly oxidized to phenol and other products, including muconic acid, which involves splitting the ring. Benzene derivatives with a single side chain usually have this side chain oxidized. Thus, toluene, or methylbenzene, and benzaldehyde are oxidized to benzoic acid.

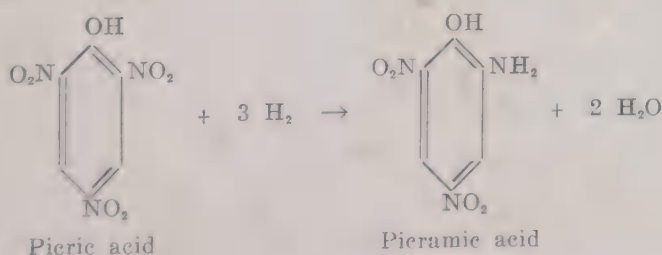


In all of these instances the final products are more acidic than the parent substance.

When two side chains are attached to the benzene ring, only one is oxidized. Compounds containing two  $COOH$  groups are oxidized with difficulty. This is very unfortunate because the aliphatic dicarboxylic acids are injurious to the kidney. The simplest member of this series, oxalic acid, is a notable example and, as is well known, may give rise to calcium oxalate crystals in the kidney and urinary tract. Oxalic acid is present in various foods, including rhubarb, spinach, chard, beet leaves, cocoa, and tea.

**Reduction.**—Reduction is less common and apparently less important than oxidation. We have seen that the bile pigments are reduced to urobilin and urobilinogen in the intestinal tract. This is generally ascribed to bacterial action; hence it can scarcely be termed a physiological mechanism. There are, however, some reduction reactions which are accomplished metabolically.

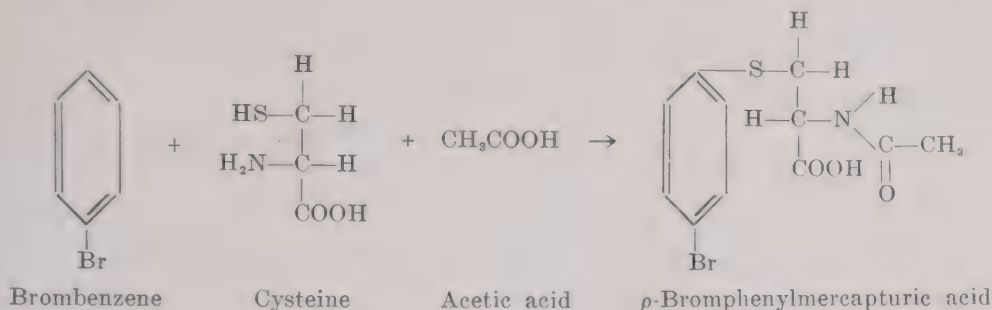
Picric acid is converted to picramic acid:





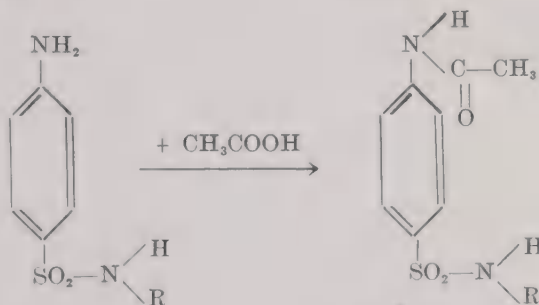


**CYSTEINE.**—Brombenzene, chlorbenzene, and iodobenzene, when fed to animals, are converted into “mercapturic acids” by conjugation with cysteine and acetylation.

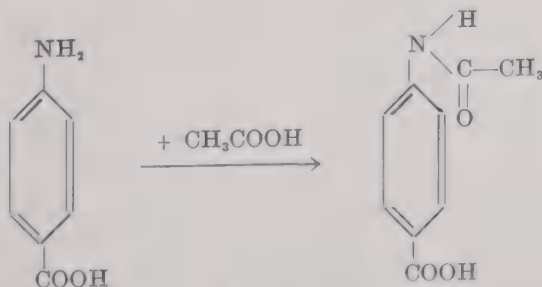


naphthalene, anthracene, benzyl chloride, and a number of other substances are known to be handled similarly by animals, and Stekol has shown that mercapturic acid formation occurs also in man. The administration of some of these poisons to animals results in an inhibition of growth if the protein intake is low. The explanation is that the cysteine required for growth is used in the detoxication process. (White and Jackson.)

**ACETIC ACID.**—It has just been seen that acetic acid is used, together with cysteine, in the formation of mercapturic acids. However, conjugation of acetic acid alone with other substances having an amino group is a very common occurrence. One notable example is the acetylation of the sulfa drugs. This occurs after absorption or parenteral administration, and their efficacy as bacteriostatic agents is thereby decreased. Coenzyme A is, of course, required for these acetylations.



As would be expected, the vitamin, para-aminobenzoic acid, is handled similarly. (Barrow.)

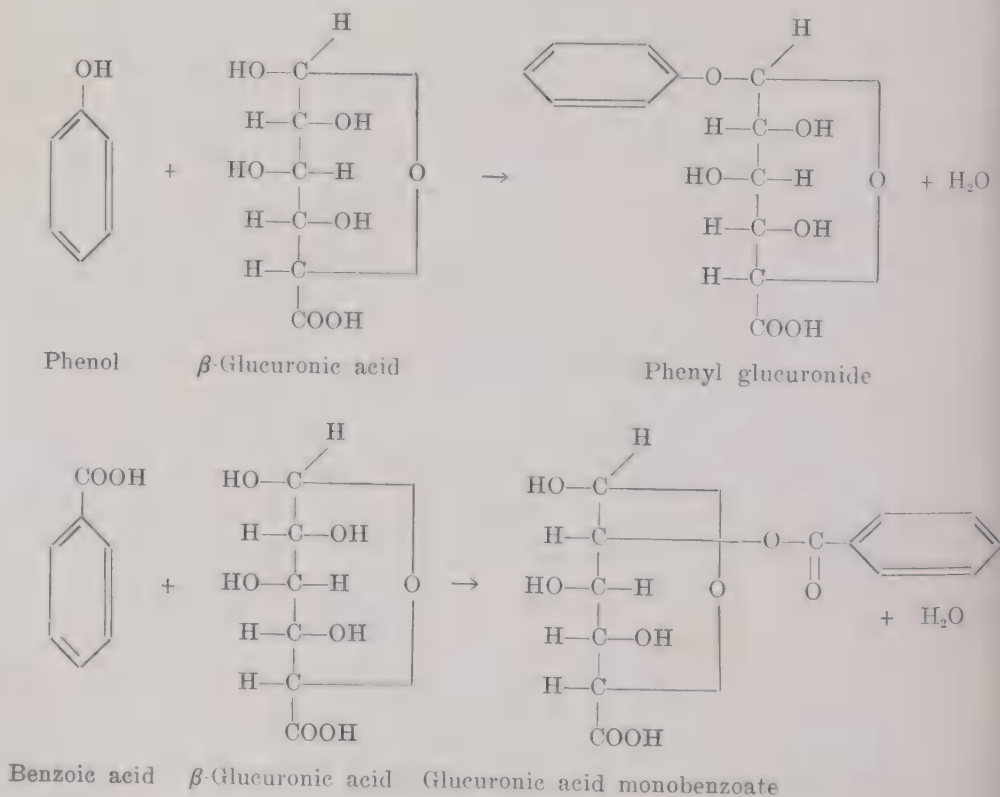


Incidentally it may be remarked that para-aminobenzoic acid (PABA) itself is a detoxicant. Symptoms of hydroquinone poisoning may be overcome by the oral

administration of the vitamin. (Martin and Ansbacher.) It also can detoxicate certain phenylarsonates, which are trypanocides, when these are given in toxic doses. (Sandground.) These actions of PABA differ from the others discussed here since they are not metabolic reactions of the cells of the body but are brought about by the *administration* of a compound, even though this is a physiological compound.

**SULFURIC ACID.**—Phenol, cresol, indole, and skatole, formed by the action of intestinal bacteria on some of the amino acids in the large intestine, are transported to the liver where they are conjugated with sulfuric acid. These processes have been followed on pages 248, 512, and 521. The resulting “ethereal sulfates” appear to be less toxic than their precursors, and, because they are more acidic, are more easily excreted by the kidney.

**GLUCURONIC ACID.**—Glucuronic acid is an oxidation product of glucose in one of the secondary paths of metabolism of glucose and glycogen. (Dziwiatkowski and Lewis.) Perhaps 150-200 mg. per day is found in the urine of a normal man. This is combined with a number of products of normal metabolism and is increased, sometimes to a considerable extent, after the administration of various drugs. The linkages with glucuronic acid are of two types, glucosidic and ester. Alcohols and phenols are combined in glucosidic linkage and acids in ester linkage. For example:



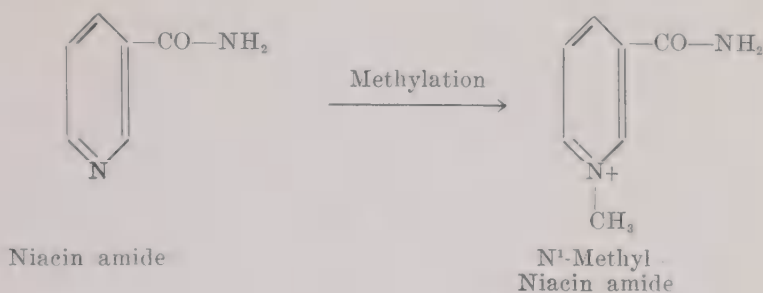
The products of sex hormone metabolism are, in a number of instances, known to be excreted as glucuronides. Morphine, menthol, camphor, chloral hydrate, borneol, salicylic acid, acetanilide, pyramidon, creosote, vanillin,



ABA, and sulfapyridine are representatives of a long list of compounds which are excreted in one or the other of these two forms.

It will be noted that several of the compounds cited, for example, benzoic acid, phenol, PABA, and the sulfa drugs, have been shown to be handled by other mechanisms, again indicating that the body uses more than one method of detoxication."

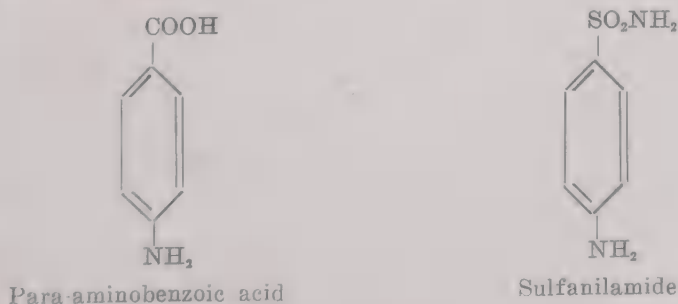
**Methylation.**—Methylation and transmethylation have been discussed on page 390, and the importance of the methyl group in the formation of methionine and creatine was indicated. While these are not detoxications in the original sense, they are syntheses, and it is known that other methylations occur in the body. Niacin is an important example. This is metabolized in part as follows (Huff and Perlzweig):



**Hydrolysis.**—Aspirin is a good example of hydrolytic action within the body. This is acetylsalicylic acid. The acetic acid formed is either oxidized or used for synthesis of physiological compounds and the salicylic acid is excreted by the kidney, combined partly with glucuronic acid. Glucosides are in many cases hydrolyzed to the sugar and the aglycone, each of which is treated by the body according to its particular nature.

## BIOCHEMICAL ANTAGONISM

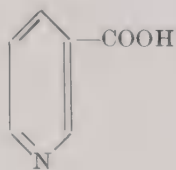
The studies of Woods on the relationship of para-aminobenzoic acid to the sulfa drugs has opened up an entirely new field in biochemistry. This is the antagonism between physiologically active compounds, such as vitamins, and certain substances having structures similar to them. These substances are either synthesized or can be obtained from natural sources. Para-aminobenzoic acid and sulfanilamide together are an example of such antagonism. Their structural similarity is apparent:



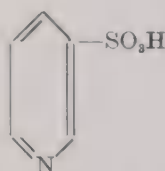
Woods found that the bacteriostatic action of the sulfonamides could be inhibited by para-aminobenzoic acid (PABA). The hypothesis is that PABA is essential for some metabolic function of the microorganisms, and that, if the sulfa drugs are present, they enter into the machinery of that function because of their similar molecular shape, but the slight difference from PABA prevents them from completely taking its place. That is, the two similar compounds compete for a place in some essential reaction, probably an enzyme system. It may be likened to a slug in a foolproof slot machine, which can enter the slot but gets caught and clogs the apparatus and can be ejected if a genuine coin comes along. The hypothesis was strengthened when it was shown that PABA was not only a cell constituent of these microorganisms, but also was essential to their growth. Furthermore, the effect of the sulfonamide is proportional to the amount of PABA present. Thus, if 50 gammas of sulfanilamide is required to prevent the growth of a strain of bacteria, having 0.01 gamma of PABA available to it, then it will require 500 gammas of sulfanilamide to counteract 0.1 gamma of PABA. In other words, the antagonism is "competitive" over a wide range of concentration of the metabolite.

Lampen and Jones have presented evidence that the primary point of sulfonamide inhibition of the growth of certain organisms is the synthesis of pteroylglutamic acid. It will be remembered that p-aminobenzoic acid forms a part of this molecule. The sulfonamides, by reason of their chemical similarity to PABA, are presumably substituted for it but are inadequate substitutes.

Based on this hypothesis a number of attempts were made to produce other bacteriostatic agents by synthesizing compounds similar to other vitamins which were known to be needed by bacteria. Among these was 3-pyridine-sulfonic acid, a "structural analogue" of niacin.

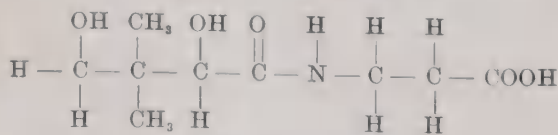


Niacin

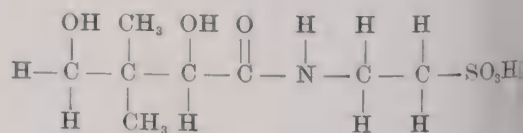


3-Pyridinesulfonic acid

3-Pyridinesulfonic acid proved to have bacteriostatic properties, as did thiopanic acid, a close relative of pantothenic acid. (Mellwain; Snell.)



Pantothenic acid



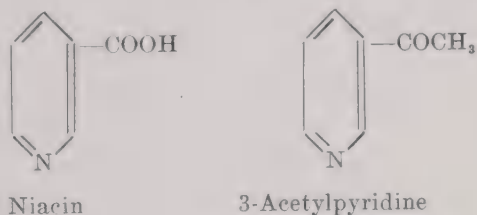
Thiopanic acid

In these cases, also, administration of sufficient amounts of the vitamin in question nullified the effects of the sulfonic acid derivative. Other vitamins for which antagonistic structural analogues have been found are pyridoxine, thiamine, riboflavin, ascorbic acid, biotin, and vitamin K.

McIlwain also showed that an  $\alpha$ -amino acid deficiency of microorganisms could be produced by feeding  $\alpha$ -amino sulfonic acids; that is, compounds differing from the physiological  $\alpha$ -amino acids in having  $-\text{SO}_3\text{H}$  groups in place of  $-\text{COOH}$  groups. Growth of the organisms could be restored by addition of  $\alpha$ -amino carboxylic acids, which did not need to correspond in structure to the inhibitor. Besides the sulfonic acids, other structural analogues of the  $\alpha$ -amino carboxylic acids have been shown to be antagonistic. For example,  $\beta$ -2-thienylalanine opposes phenylalanine. (du Vigneaud.)



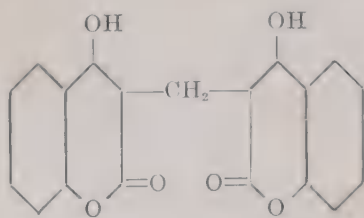
All of these effects were on microorganisms, and it will be noted that they really amount to producing vitamin (or amino acid) deficiencies in these lower forms. However, Woolley and associates in 1938 had shown that 3-acetylpyridine, resembling niacin in chemical structure, possessed no vitamin activity when included in the diets of mammals and, in fact, was poisonous to them.



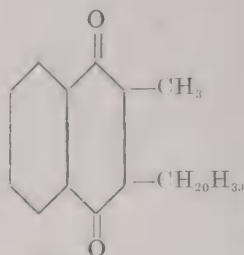
The 3-acetylpyridine, by taking the place of niacin, in some vital enzyme reaction had "clogged" that reaction. Other attempts were made to produce vitamin deficiencies by adding structural analogues to the diet, and, even in the presence of the otherwise requisite amount of the real vitamin, these attempts were frequently successful. One such experiment resulted in the production of pantothenic acid deficiency in mice by long-continued feeding of thiopanic acid (pantoyltaurine), a structural analogue of the vitamin. (Snell.) Similarly, 2-hydroxy-1,4-naphthoquinone was found to be a competitor to vitamin K. It produced a fatal hemorrhagic syndrome due to a reduction in the level of plasma prothrombin. Vitamin K, it will be remembered, stimulates the formation of prothrombin, and administration of it in these experiments tended to counteract the action of the compound mentioned. (Smith.) The action of Dicumarol in producing a decline in prothrombin level may also be regarded as an antagonism to vitamin K. The resemblance of their structures is apparent. However, large doses of the vitamin are needed to overcome the effects of dicumarol, while only minute amounts are ordinarily needed to cure avitaminosis K. This is quite different from the quantitative relations described



for PABA and sulfanilamide and has been termed "noncompetitive antagonism."

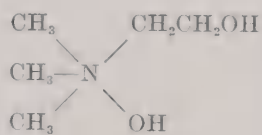


Dicumarol

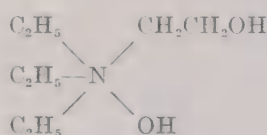


Vitamin K

Several derivatives of pterylglutamic acid have been found to inhibit its action. The most active ones are those containing the 4-aminopteroylglutamyl moiety, and of these the most potent is aminopterin; as little as 20  $\gamma$  will kill a weanling rat in a few days. (Oleson.) Choline may be antagonized by its triethyl analogue, "triethylcholine."



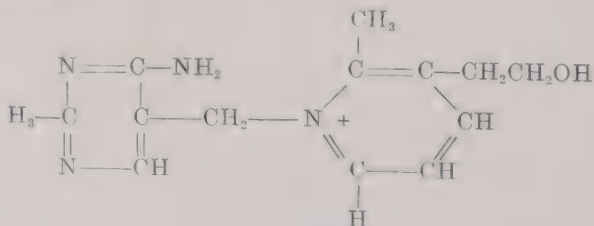
Choline



"Triethylcholine"

In sufficient doses the triethyl derivative produces muscular weakness and convulsions. Preliminary injection of choline protects against this toxic effect. The interpretation is that "triethylcholine" interferes with acetylcholine formation because of its structural similarity. Curiously, however, the triethyl compound can actually substitute for the trimethyl in another physiological function; namely, lipotropic action. (Keston and Wortis.)

Structural analogues of the vitamins are sometimes called "antivitamins," and the ratio of the amount of antivitamin needed to combat the effective amount of vitamin is the "inhibition ratio." This is high in most cases. Thus, for pyrithiamine, a thiamine antagonist, the ratio is approximately 40:1; that is, 40 moles of pyrithiamine are required to counteract the effect of 1 mole of thiamine. Pyrithiamine, which has a  $-\text{CH}=\text{CH}-$  group in the place of the  $-\text{S}-$  group of thiamine, causes a thiamine deficiency when fed to mice. (Woolley and White.)

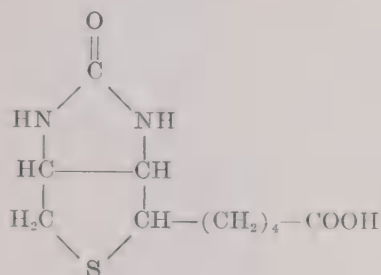


Pyrithiamine

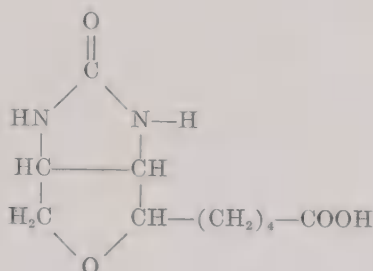
Perhaps the most potent antivitamin reported is desoxypyridoxine which has an inhibition ratio of 2:1 in the chick.

As a result of all these investigations one would expect to find that many new chemotherapeutic agents would be available. That is, if these structural analogues interfere with the growth of microorganisms because they block essential reactions in the cell, should they not be useful as drugs? Thus far very few have been found to be of value in this respect. The sulfonamides are the notable exceptions. The reasons vary with the individual substances. Some of the inhibitors are as harmful to the host as to the invader. For some the concentration required for inhibition is too great, or the essential metabolite which is to be inhibited is required in too large amounts. Others are excreted or destroyed too rapidly.

Sometimes the modification of the structure of a vitamin produces a new vitamin with the same activity, rather than an antivitamin. Such a result occurred when oxybiotin was formed by the substitution of oxygen for sulfur in biotin. (Pilgrim.)



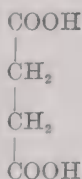
Biotin



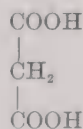
Oxybiotin

The search for compounds which will overcome these objections continues, however, with great intensity.

These biochemical antagonists are believed to produce their effect because of their resemblance to some part of the enzyme system, that is, a part of the enzyme itself, or of a coenzyme. There are also *substrate* analogues which compete for the enzyme systems. Malonate, for example, can inhibit succinic dehydrogenase because it competes with succinate for this enzyme.



Succinic acid



Malonic acid

A similar case is the decarboxylation of tyrosine by a decarboxylase. This is considered to be a step in the production of epinephrine, which, it will be remembered, causes a rise in blood pressure. Structural analogues of tyrosine and of dihydroxy-L-phenylalanine specifically inhibit the enzyme and lower blood pressure in the intact animal. (Martin, 1950.) This is an interesting approach to therapy in hypertension.

It must be remembered that not all structural analogues of vitamins, amino acids, or other physiological compounds are inhibitors of enzyme systems and

therefore antagonists. Most of them, indeed, are inert. It is the interesting exceptions which have this remarkable property. One cannot predict how a structural analogue will behave. For example, the substitution of oxygen for sulfur in biotin cited above did not lead to a metabolic antagonist, although the similar substitution in methionine produced an effective substance, methoxinine. (Roblin.)



Methionine



Methoxinine

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## Chapter 25

### RECENT CLINICAL APPLICATIONS

#### LIVER FUNCTION TESTS

Many attempts have been made to devise a test which would measure, with some degree of accuracy, the amount of normal liver tissue actively functioning. Two facts have militated against such an achievement. First, although the liver may be quite badly damaged, it may nevertheless perform all its functions, because only a comparatively small proportion of healthy liver tissue is needed for all normal activities. That is, the liver has a large "factor of safety." Second, the functions of the liver are many and diverse in nature. Liver is concerned in protein, carbohydrate, and fat metabolism, in the production of the plasma proteins and heparin, in the secretion of bile, in storage, in "detoxication" and excretion, and in a number of other activities. It often happens that a defect in performing one function is not paralleled by a diminution in others, and the function tested may happen to be one that is not affected.

Nevertheless, if one knows their limitations, certain of these tests have clinical value. Some of the procedures will be outlined, giving their biochemical background when possible.

**Icteric Index and Van den Bergh Tests.**—These have been described on page 597. They furnish information regarding the concentration of bile pigment in blood serum. The modifications of the Van den Bergh test also indicate whether the pigment is in a combined or uncombined state. These tests, together with the determination of urobilinogen and bilirubin in the urine, aid in the differential diagnosis of obstructive and nonobstructive jaundice.

**Tests of Carbohydrate Function.**—These tests are based on the ability of the liver to utilize sugars. Since glucose is very easily handled, other monosaccharides, which are less easily converted into glycogen by the liver, are used. Thus fructose or galactose, when administered in comparatively large amount, puts a strain upon the liver. If the former is used, the blood sugar is followed as in a glucose tolerance test. With galactose, the excretion in the urine is determined. Normally 3 Gm. or less are eliminated in five hours following the administration of 40 Gm. of galactose. If there is liver damage, from 4 to 5 Gm. or more are excreted.

**Hippuric Acid Test.**—This procedure has been described on page 520. It tests the ability of the liver to conjugate certain compounds, such as benzoic acid, with glycine.

**Dye Secretion Tests.**—There are several methods in which is employed a nontoxic dye that is excreted practically exclusively by the liver. The amount excreted, and hence the functional capacity of the liver to eliminate

it, may be found by determining the amount still circulating in the blood after a definite length of time. Bromsulfalein is the dye most generally employed. It is injected intravenously, and one or two samples of blood are taken at specified times and the serum analyzed colorimetrically. The presence of more than 50 per cent of the dye after five minutes, or any dye at all after forty-five minutes, is evidence of hepatic insufficiency.

**Cephalin-Cholesterol Flocculation Test.**—In 1939 Hanger showed that emulsions of mixtures of cephalin and cholesterol are not flocculated by normal serum or by serum from patients having obstructive jaundice. They are flocculated by sera from patients having active disturbances of the hepatic parenchyma. It is therefore looked upon as an index of parenchymatous function only. The test is said to depend upon the capacity of gamma globulin in serum to unite with the colloidal constituents of the emulsion, to produce flocculation. Normally the serum albumin inhibits this action of gamma globulin, but in cases of parenchymal liver disease, the serum albumin has a decreased power to inhibit the globulin. (Moore.)

**Alkaline Phosphatase.**—Alkaline phosphatase is excreted normally in large amounts into the bile, and hence this is assumed to be a function of the liver, and the concentration of alkaline phosphatase in blood, an index of this function. However, it should be remembered that this enzyme is concerned in bone metabolism and fluctuations in its level in the blood occur in bone diseases. Therefore it is not surprising to note that observers have insisted that the alkaline phosphatase should not be considered alone but always in conjunction with at least one other functional test. Thus, a high cephalin-cholesterol flocculation with a low phosphatase may indicate hepatitis with no duct obstruction, whereas a high phosphatase with a low cephalin-cholesterol flocculation is likely to mean an obstruction without hepatitis.

**Composite Test.**—A number of other tests have been described, and some of them are based on considerations similar to those just described. Among them are a colloidal gold test, prothrombin determination, blood protein determination, etc. McGavack and his co-workers recommend a group of procedures, namely, the icteric index, Van den Bergh reaction, cephalin-cholesterol flocculation, alkaline phosphatase, total cholesterol and cholesterol esters, total plasma proteins, and the albumin:globulin ratio. The advantages of this "composite test" are: (1) all the tests can be done on a single specimen of blood serum; (2) the renal factor is ruled out; and (3) a number of liver functions are evaluated simultaneously. As a consequence, it is stated that fairly characteristic pictures are obtained in several hepatic conditions.

## KIDNEY FUNCTION TESTS

The body has a considerable factor of safety in renal, as well as hepatic, tissue. One normal kidney can do the work of two, and, if all other organs are functioning properly, less than a whole kidney may suffice. On the other hand, there are extrarenal factors which interfere with kidney function, particularly circulatory disturbances. It is therefore very important to have methods which appraise the functional capacity of the kidneys. Such tests have been devised



but, as in the case of liver function tests, no single test can measure all the kidney functions, although the kidney is not as versatile an organ as the liver. Consequently more than one test is usually indicated. It must also be remembered that these procedures throw light upon the functional capacity of the kidney as related to the general physiology of the patient and not upon the extent of any lesion or pathological process. Many renal function tests have been proposed and are being used, but only a few can be given space here.

Some of the procedures used have been discussed in other chapters. A study of the nitrogen retention has been considered in Chapter 22. It will be remembered that a stepwise increase in three nitrogenous constituents of blood is believed by some to parallel a deteriorating kidney function. Uric acid usually rises first, later urea, and finally creatinine. By determining all three, an estimate of kidney function may be made. It must be remembered, of course, that gout and certain other conditions result in a high uric acid also. Glucose, or, at any rate, a substance reacting like glucose, is often increased in the blood in nephritis and there may be other changes as well.

The concentration and dilution tests for renal function have been briefly described on page 507. There are a number of variations but all are based on the principle that the normally functioning kidney is capable of secreting a dilute urine if a large volume of fluid has been ingested and a concentrated urine if the individual has been deprived of fluid.

**Phenolsulfonephthalein Test.**—Phenolsulfonephthalein is a harmless dye which, after parenteral administration, is eliminated only by the kidneys. It is easily detected and estimated by colorimetric methods. Under standard conditions it appears in the urine normally in about ten minutes, and, within the first hour thereafter, from 40 to 50 per cent are eliminated; in two hours, a total of from 60 to 70 per cent. In renal insufficiency the amount secreted in two hours is much reduced, sometimes to even a trace. In very early nephritis an excessively high elimination may be found, owing to irritation or to a compensatory hyperactivity of undamaged kidney tissue. In such cases other tests must be used to aid in diagnosis. The phenolsulfonephthalein test is widely used, because it is easy to perform, and has given very valuable information in many instances.

**Blood Urea Clearance Test.**—The concentration of urea in the blood rises in nephritis and in other conditions of deficient kidney function. However, as has been mentioned previously, this is subject to great fluctuations and is not, by itself, a good index of the ability of the kidney to excrete nitrogenous waste. Ambard was the first to study the concentration of urea in the blood and relate it to the rate of excretion in the urine, and "Ambard's coefficient" was for a while the subject of much clinical study. At present the blood or, better, the plasma urea clearance test of Van Slyke and his colleagues is widely used. By "plasma urea clearance" is meant the rate at which plasma is cleared of urine while passing through the kidneys. As a matter of fact, the plasma is not completely cleared of urea. Only about 10 per cent of the urea is removed. Consequently, if 750 ml. of plasma pass through the kidney per minute, and 10 per cent of its urea is removed, it is equivalent to completely clearing 75 ml. of plasma per minute.

The data required are the plasma urea concentration, the urine urea concentration, and the rate of urinary flow. When the volume of urine secreted is large, the rate of urea excretion is directly proportional to the concentration of urea in the blood. When this volume is small, this simple relationship does not hold. Therefore, two different formulas are required for calculating the urea clearance.

A. Maximum clearance ( $C_m$ ), when 2 ml. or more urine is secreted per minute:

$$C_m = U/B \times V$$

B. Standard clearance ( $C_s$ ), when the urinary secretion amounts to less than 2 ml. per minute:

$$C_s = U/B \times \sqrt{V}$$

Where  $U$  = mg. urea N per 100 ml. of urine

$B$  = mg. urea N per 100 ml. of plasma

$V$  = urine volume in ml. per minute

The technique of a typical test, with illustrative figures, is as follows:

7:00 A.M. Patient receives a light breakfast; later, one glass of water

9:00 A.M. Bladder emptied and urine discarded

9:50 A.M. Sample of blood taken

10:00 A.M. Bladder emptied and urine saved

11:00 A.M. Bladder emptied and urine saved

(Exact one-hour specimens are not necessary, but the exact periods to which they correspond must be known.)

Plasma urea N: 15 mg. per 100 ml.

First urine specimen: 48 ml., 770 mg. urea N per 100 ml.

Second urine specimen: 52 ml., 730 mg. urea N per 100 ml.

Average 50 ml., 750 mg. urea N per 100 ml.

Since the volume of urine is 50 ml. per hour or 0.83 ml. per minute, the standard formula is used:

$$C_s = 750/15 \times \sqrt{0.83} = 45.5 \text{ ml. of plasma cleared of urea per minute}$$

The average normal  $C_s$  = 54 ml. of plasma cleared per minute. Therefore this case showed 45.5/54 or 84 per cent of normal. A figure of 75 per cent or more of "normal" is considered normal. In maximum clearances, the average normal is 75 ml. of plasma cleared per minute.

**The "Urea Ratio."**—In 1917 Mosenthal and Hiller suggested the ratio of urea nitrogen to the nonprotein nitrogen of the blood as an index of effectively functioning renal tissue, irrespective of the level of the blood urea. More recently Mosenthal and Bruger have carried out a long series of determinations and have shown that this ratio parallels rather closely the figures for blood urea clearance. The "urea ratio" is  $100 \times \text{urea nitrogen/nonprotein nitrogen}$ . With normal renal function the urea ratio is 44 or less; with a maximal impairment, 80 or higher; with improvement the ratio falls, and with progressive impairment it rises. In eclampsia the index is definitely lower.

The advantages of this method are that it requires but one sample of blood, does not demand prolonged observation of the patient or collection of urine, and furnishes a numerical index of the degree of impairment of renal function.

**Inulin and Diodrast Clearance Tests.**—Inulin and diodrast have been used in clearance tests because they are selectively secreted. Inulin is removed from the blood only by the glomeruli, while diodrast is excreted almost entirely by the tubules. Inulin, it will be remembered, is the polysaccharide yielding fructose on hydrolysis. Diodrast is a complex organic iodine compound (3,5-diiodo-4-pyridine-N-acetic acid diethanolamine). It is opaque to roentgen rays and is used for roentgenological examination of the urinary tract. By determining the amount excreted and the plasma content under standard conditions, either the inulin or the diodrast clearance can be determined, and thus the functional activity of the glomeruli or tubules may be estimated. By doing simultaneous diodrast and inulin clearance tests, Smith maintains that the active mass of renal tissue, the "tubular excretory mass," can be ascertained.

**Pancreatic Function Test.**—The fact that secretin stimulates the flow of pancreatic juice has been made use of in a test of external pancreatic function. (Agren.) The preparations now available are free from histamine, cholecystokinin, and many other contaminants but are really mixtures of secretin and pancreozymin. A double lumen tube, with sections of unequal length, is passed so that the longer end reaches the third portion of the duodenum and the shorter end remains in the stomach. Continuous aspiration with a negative pressure of 20-30 mm. of mercury prevents the overflow of gastric juice into the duodenum and sucks out both gastric juice and duodenal contents into separate containers. After a basal flow has been obtained, the secretin is injected intravenously and one measures volume of flow and bicarbonate concentration. Sometimes the enzymes are also determined.

Shortly after the injection there occurs an outpouring of pancreatic juice. The duodenal fluid, therefore, loses its biliary color under normal conditions, but if this bile color remains, a nonfunctioning gall bladder is indicated. The total volume varies normally from 135 to 250 ml. in one hour, and the bicarbonate, from 90 to 130 meq.

The test is of value in detecting disease of the pancreas when all other tests have failed. In pancreatitis with extensive destruction of parenchymal structures, there is usually a diminution in the volume and bicarbonate output. In less severe pancreatitis about half the cases show these effects. (Dornberger.) The influence upon the enzymes has not been constant enough to justify their determination. In pancreatic malignancy there is a lowering of the volume response with less change in the bicarbonate. (Dreiling and Hollander.)

## BLOOD PRESSURE

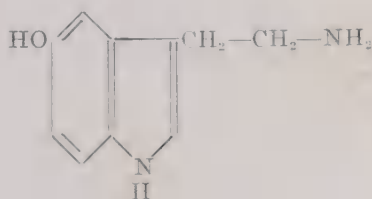
Goldblatt demonstrated some time ago that mechanical interference with the blood flow through the renal arteries of a dog resulted in the development of a permanent hypertension. Apparently, slowing the circulation causes some substance, which produces vasoconstriction, to be formed. Such a substance has been demonstrated in the blood coming from such "ischemic" kidneys. The mechanism seems to be the following: An enzyme, "renin," is formed in the kidney and is released into the blood. It is a proteinase and acts upon an  $\alpha_2$  globulin, present in the systemic blood. A product of its activity is a polypeptide, "angiotonin," which has a molecular weight of about 2,700 and is



thermostable and dialyzable. Angiotonin constricts arterioles and increases the force of the heartbeat, resulting in increased arterial pressure. (Page and Helmer.) Another proteolytic enzyme, a peptidase, is produced by the kidney. This destroys angiotonin and is thus an antipressor factor. Various investigators have designated these factors by different names. Thus, the  $\alpha_2$  globulin has been called "renin activator," "hypertensinogen," and "renin substrate"; the polypeptide, angiotonin, has been termed "hypertensin"; and the inactivating peptidase, "angiotonase," "angiotoninase," and "hypertensinase." It must be said that, thus far, renin has not been found in excess in patients with chronic renal hypertension. According to Shorr, the ischemic kidney also forms, in its cortical portion, vasoexcitor material (VEM). This may act as a neutralizing agent for VDM, the hepatic vasodepressor material, which has been shown to be identical with ferritin. (See also page 481.)

Substances which lower blood pressure have also been obtained from kidney extracts. They have not been isolated in pure form but give promise of therapeutic usefulness in cases of hypertension. (Jablons.)

It has long been known that after blood coagulates it possesses vasoconstrictor properties. In 1948 the active agent was isolated and crystallized by Page and his group and called "serotonin." It was analyzed and found to be a complex of creatinine, sulfuric acid, and 5-hydroxytryptamine. Since the pharmacologic properties reside in the 5-hydroxytryptamine, which is separable from the complex, the name "serotonin" has been assigned to it. (Rappoport.) It has also been synthesized. (Hamlin and Fischer; Speeter.) Serotonin causes other types of smooth muscle to contract and may prove to be an important physiological agent. Antimetabolites of serotonin have been produced. (Woolley and Shaw.)



Serotonin

## DENTAL CARIES

Dental caries is one of the most widespread of human diseases, and a tremendous amount of investigation has been instituted to determine the cause and effect a cure. The results up to the present time have been rather conflicting. In caries the enamel and other hard structures are dissolved by chemical action and washed away, thus producing a cavity. The formation of cavities in the teeth is not only a source of pain and discomfort, necessitating dental attention, but it is likely to lead to interference with mastication, and hence with proper nutrition. Furthermore, infectious processes occurring in the cavities may result in the absorption of toxins or lead to secondary infections in other parts of the body.

In general, there are two schools of thought relating to the initiation of caries. There are some who believe that local factors are entirely or, at any

rate, chiefly responsible. Food particles lodging between the teeth, or in recesses in the surface of teeth, become breeding spots for bacteria. If they are not removed promptly, enough acid is produced to dissolve the mineral constituents of the enamel and dentin. Foods, such as soft cereals, candies, and pastries, are most easily fermented and consequently are most likely to lead to the formation of cavities. (See page 139.) Although saliva has no bactericidal power, the character of the saliva is believed to play a role. The more mucin it contains, the less effective it is in cleaning the teeth. The adherents of this "chemico-parasitic" theory advocate oral hygiene and prophylaxis as a deterrent to caries. The possibility of a proteolytic factor has attracted considerable attention. According to this view, caries is a proteolytic process, or perhaps the proteolytic and glycolytic processes go on side by side. (Gottlieb.) It is assumed that bacteria, possessing proteolytic enzymes, multiply at the surface of the teeth and cause a disintegration of the lamellae, the flattened bands of organic protein-containing matter extending through the enamel. This permits the easy entrance of the fermenting organisms with their production of acid, and consequent solution of the inorganic portion of the enamel.

The other view is that although caries is a local action and always begins at the exterior of the tooth, the structure of the tooth determines whether or not decay will occur. An excellent nutritive condition of the individual is responsible for perfection of the structure, and since the formation of teeth begins in fetal life, the food of the mother is just as important as that of the child. The vitamins A, C, and D, and the elements calcium and phosphorus, with traces of fluorine, are all considered essential for the building of healthy teeth.

The influence of fluoride, which has been discussed on page 483, must be emphasized. When fluoride is ingested over a long period of time and in considerable quantities, a condition known as "mottled enamel" occurs. This enamel is not only discolored, but also brittle. Caries can and does occur in such teeth. However, if the amount of fluoride is less, i.e., not sufficient to cause mottling, the enamel seems to be *more resistant* than normal and the development of dental caries is less likely to occur. It should be noted that fluoride is only effective if it is present during the period of tooth development. There are several explanations for the possible inhibiting action of fluoride on caries. One is that fluorine is an essential, or at any rate a highly desirable, component of enamel; that is, that fluorine reacts with the tooth substance to form a less soluble complex, a compound less susceptible to the solvent action of acids. Support of this hypothesis has recently been offered. Using isotope exchange and ion competition techniques, it was found that fluoride can replace hydroxyl or bicarbonate ions on the surface of bone, forming a very insoluble and resistant "fluoroapatite." It is suggested that the same phenomenon occurs in the mouth. (Neuman.) A second hypothesis is that the fluorine acts as an enzyme inhibitor, thus interrupting the chain of fermentative reactions and preventing the formation of the organic acids in close proximity to the enamel. The use of fluoride in the water supplies of communities is being tested as a possible method of preventing dental caries. In this connection it is interesting to note that the hardness of drinking water may also play a part. Mills states that there is a lower incidence of carious



lesions in regions where the drinking water is hard than in those in which it is soft, and this has been substantiated. Whether this is a nutritional effect of the additional calcium or magnesium present, or a local effect, is not apparent.

## BIOCHEMISTRY OF INFLAMMATION

Menken has presented evidence that the various stages of inflammations are referable to chemical entities, all of them of protein nature. An inflammation is a manifestation of severe cellular injury. As a result it is assumed that there is produced a substance which causes increased capillary permeability. This is a polypeptide, to which there may be attached a prosthetic group. It has been called leucotaxine and has been found in exudative material. It is quite a different substance from hyaluronidase, and, of course, from histamine, acetylcholine, or adenine derivatives.

Leucocytosis is caused by another factor, which also has been isolated from exudates. This substance is a protein, probably a part of the pseudoglobulin fraction. The leucocytosis-promoting factor, or LPF, induces a discharge into the circulation of immature granulocytes.

Since inflammation is always accompanied by a certain amount of breakdown of tissues, it is not surprising to find that a proteolytic enzyme is involved. This seems to be liberated from cells which have been initially injured by an irritant and has been termed "necrosin." This is a toxic euglobulin and can also be found in exudates. When injected into the skin it causes swelling, redness, necrosis, lymphatic blockade, injury to the blood vessel walls, and swelling of collagenous bundles.

A pyrogenic substance, called "pyrexin," can also be obtained from exudates. This is thermostable and may possibly be a glycopeptide. It can raise the temperature of an experimental animal into which it has been injected by 2 or 3° F. Closely associated with pyrexin is a leucopenic factor, probably a polypeptide. The occurrence of such a substance may serve to explain the leucopenia attending certain inflammatory conditions; for example, influenza and typhoid fever. Of course this has an action antagonistic to the LPF. Finally, there is thought to be liberated from the injured cell one or more growth-promoting substances responsible for eventual repair.

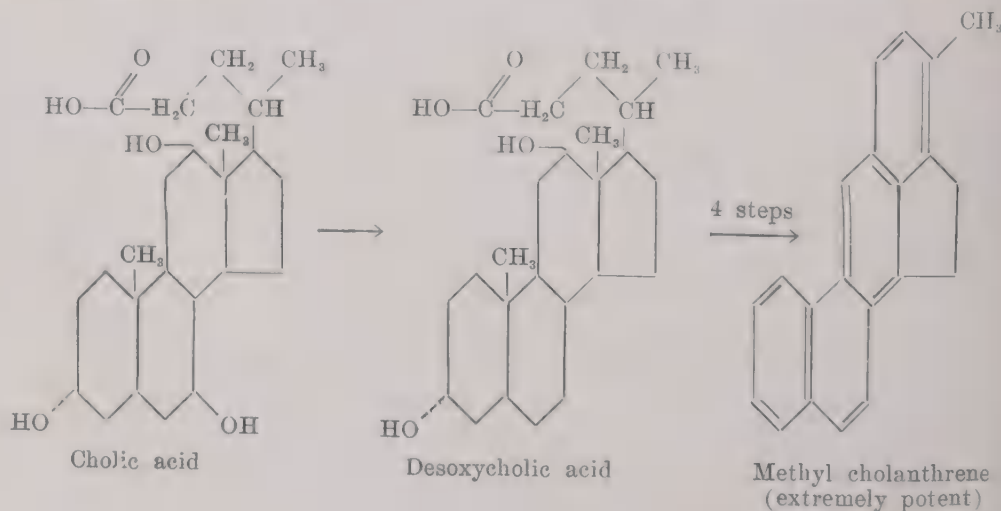
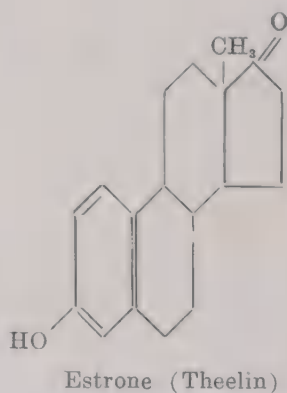
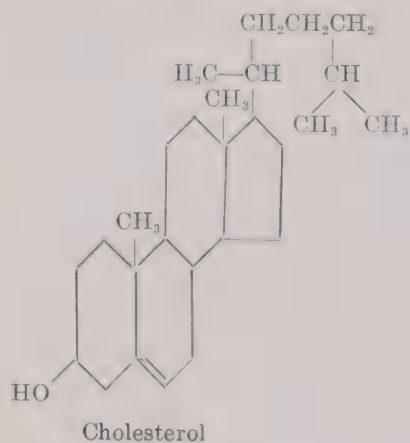
## THE BIOCHEMISTRY OF TUMORS

Tumor growth, that is, the "wild" growth of cells, has a number of biochemical aspects. It is possible that it is caused by some toxic substance arising in normal metabolism which is ordinarily detoxicated or completely eliminated. It is also possible that unusual conditions of life modify the normal biochemical processes, so that a cancer-producing, or carcinogenic, substance is formed. A further possibility is a change in the normal enzyme reactions, resulting in a distorted distribution of energy, and consequently of growth. Thus marked trends in cancer investigations have been the search for chemical etiological factors, and different patterns of enzyme systems. But biochemistry may also be involved in diagnosis because sometimes the growth of a tumor markedly changes the normal chemistry of the body. The following discussion will

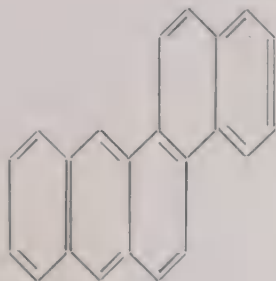


serve to illustrate some applications of biochemical studies to this complex field of pathology.

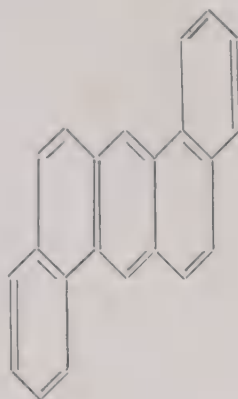
In 1775, Pott drew attention to the fact that chimney sweeps frequently had cancer of the scrotum, a result of the contact of the abraided skin with chimney soot. This was later correlated with the coal tar, which was definitely established as a substance capable of causing cancer in man. In the early part of the present century it was shown that frequent application of gasworks tar to the rabbit's ear produced cancer. Following this, Cook and his co-workers investigated the effects of many organic compounds, some of which were present in tar but many of which were not found in nature or industrial products. Soon other laboratories joined in the search, and now many definite chemical compounds are known which have carcinogenic properties. At present no definite relationship can be shown between chemical constitution and cancer-producing properties. The only active compound which has been isolated from coal tar is benzpyrene. One of the most powerful carcinogens thus far discovered is methyl cholanthrene. This is particularly interesting from a biochemical standpoint, because it has been synthesized by Fieser, using cholic acid as a starting point. Cholic acid, it will be remembered, is a bile acid, as well as a part of the molecule of the other bile acids. The relationship of this to cholesterol, and to one of the sex hormones, is shown in the series of formulas below :



The three most powerful carcinogenic substances thus far discovered are methylcholanthrene, cholanthrene, and 1,2-benzpyrene.



1,2-benzpyrene



1,2,5,6-dibenzpyrene

Some very instructive studies have been carried out with 1,2,5,6, dibenzpyrene, which is also carcinogenic. Definite amounts of this, incorporated in cholesterol pellets, were inserted under the skin of experimental animals. After tumors had appeared, the pellets were removed, and upon analysis, minute amounts of the causative agent were found in the tumors, but after transplanting them repeatedly, they were found to be still cancerous in the fifth or sixth generation, at which point the tissue contained none of the originally used substance. In other words, although these toxic substances *initiate* tumor growth, the cells themselves are cancerous and continue this unrestrained habit of growth independently.

It has also been found that some of the carcinogenic compounds are estrogenic when suitably tested. On the other hand, in mice and guinea pigs the estrogenic hormones may produce cancer. The latter effect does not hold for all species. It apparently does not occur in human beings, since thousands of parenteral injections of estrogens are made daily and no increase in cancer incidence has been reported. However, one interesting development in the study of prostatic tumors relates to another sex hormone. The normal development of the prostate gland apparently depends upon hormones elaborated by the testes. The prostate regresses and atrophy of the epithelium occurs when castration is performed. In animals under such circumstances, administration of the male hormone causes regeneration of the prostate. These facts led to the idea of treating prostatic tumors by deprivation of male hormones. This has been tested by two methods, the surgical removal of the testes, or the administration of estrogens, since, in a very general way, estrogens and androgens are antagonistic. The results have been favorable in a number of instances, and again we have some evidence of the biochemical nature of the factors involved in tumor causation and possible tumor control.

Species differences also hold for some of the carcinogenic compounds already discussed. None of the synthetic compounds seem to affect primates, and rabbits are almost immune. It is possible that some mechanism for detoxicating them is operative. In the rabbit, dibenzanthracene is hydroxylated and ex-

creted in the urine. When this dihydroxy compound is administered to a mouse, which is susceptible to dibenzanthracene, no tumor results. If mice could hydroxylate the compound, they also, presumably, would be immune to its effects.

That vitamins and enzymes may be concerned in protection against carcinogenic compounds has come out of the studies of Rhoads, du Vigneaud, and others. A dye, para-dimethylaminoazobenzene or "butter yellow," will produce cancer of the liver in rats on a deficient diet. Supplements of yeast and casein to such diets have a pronounced protective action. This led to a search for one of the B complex vitamins as one of the effective anticarcinogenic agents, the other being presumably, a peptide. Riboflavin was found to be quite potent, especially in combination with casein. Another possibility was biotin, but it appears that biotin has just the opposite action. That is, when biotin is added to a highly protective diet, butter yellow is more potent than without it. Biotin is "procarcinogenic."

**The Chemistry of Tumor Tissue.**—Greenstein makes the following generalizations regarding the chemical pattern of normal tissues and of transplanted tumor tissues.

"(a) Each normal tissue is characterized by the possession of an individual pattern of enzymic activity which may serve to distinguish it from all other tissues."

"(b) Tumors have qualitatively the same enzymes as normal tissues."

"(c) The enzymatic pattern of a tumor is largely independent of its age, of its growth rate, and of the strain of animal in which it is grown."

"(d) The range of activity of each enzyme and of concentration of such components as the vitamins is much narrower among tumors than among normal tissues, *i.e.*, tumors possess a more uniform and less diverse chemical pattern than normal tissues."

"(e) When a normal tissue becomes neoplastic many of the specific functional activities markedly decrease or are lost altogether."

"(f) The range of values for the tumors is usually between the extremes of the corresponding values for normal tissues. . . . It cannot be said, therefore, that 'tumors are lower (or higher) in activity than normal tissues,' but only that their activity is lower (or higher) in respect to certain specified normal tissues. Tumors do not stand outside the metabolic range of normal tissues."

Thus mouse and rat tumors have a relatively elevated content of dehydropeptidase I, benzoylarginineamidase, and xanthine dehydrogenase, and a diminished activity of catalase, cytochrome oxidase, alkaline phosphatase, esterase, cystine desulfurase, and dehydropeptidase II.

It should be emphasized, however, that, as yet, no biochemical representation of the essential nature of cancer tissue has been defined. In addition, there is evidence of considerable biochemical and biological variation between individual cancer tissues of the same sites of origin.

**Industrial Factors in Human Carcinogenesis.**—There has been a considerable rise in the number and variety of occupational cancers. (Heuper.) This parallels the enormous modern industrial expansion. Many of the agents and



Physical factors causing them are known, but it is safe to assume that many more are still unknown, particularly those carcinogens of low potency. A partial list of "environmental carcinogens" and their sites of action in man follows: anthracene (crude), arsenic, burns (thermic), mineral oil (crude),

### SYSTEMIC EFFECTS OF MALIGNANT TUMORS

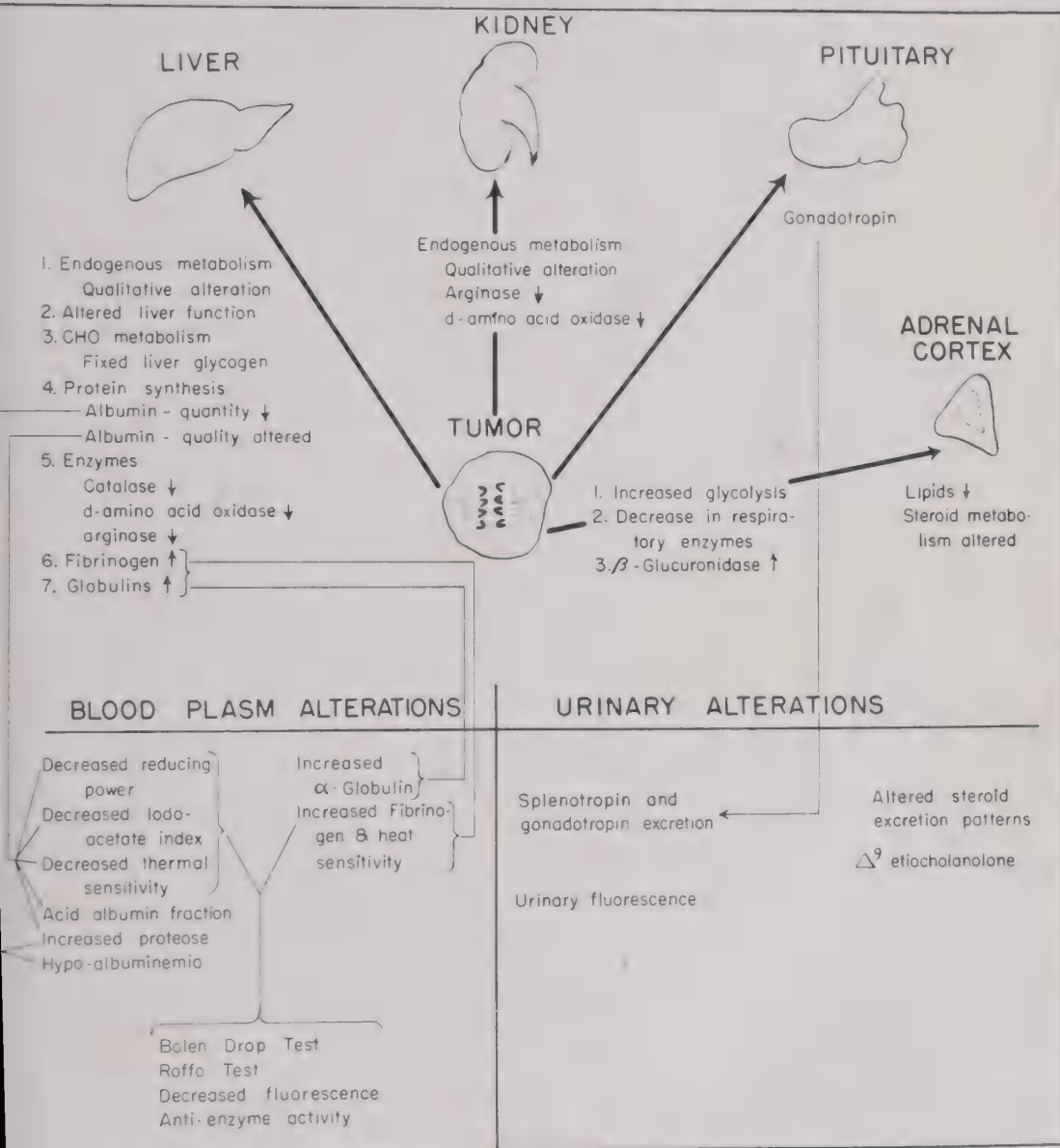


Fig. 91.—Systemic effects of malignant tumors. (From Black, M.M., and Speer, F.D.: *Am. J. Clin. Path.* 20: 446, 1950.)

paraffin oil (crude), pitch, radioactive substances, roentgen rays, soot, spindle oil, tar, ultraviolet light—all acting on the skin and appendages; chromates and radioactive substances, affecting the respiratory system; naphthylamine (beta) and schisteoma, affecting the urinary system; benzol, acting on the reticuloendothelial system; radioactive substances and roentgen rays, also act-

ing on the reticuloendothelial system and on mesenchymal tissue; and arsenic, crude mineral oil, pitch, and ultraviolet light, affecting the eye and surrounding tissues.

**Systemic Changes in the Cancer Host.**—A wide variety of systemic alterations occur in the host, coincidentally with malignant neoplasia. Some of these changes are depicted in Fig. 91. While these changes are not specific for cancer, they do provide evidence of the complexity of the systemic alterations involved and indicate the need for a more adequate study of cancer as a systemic disease.

### ACID PHOSPHATASE AND THE PROSTATE GLAND

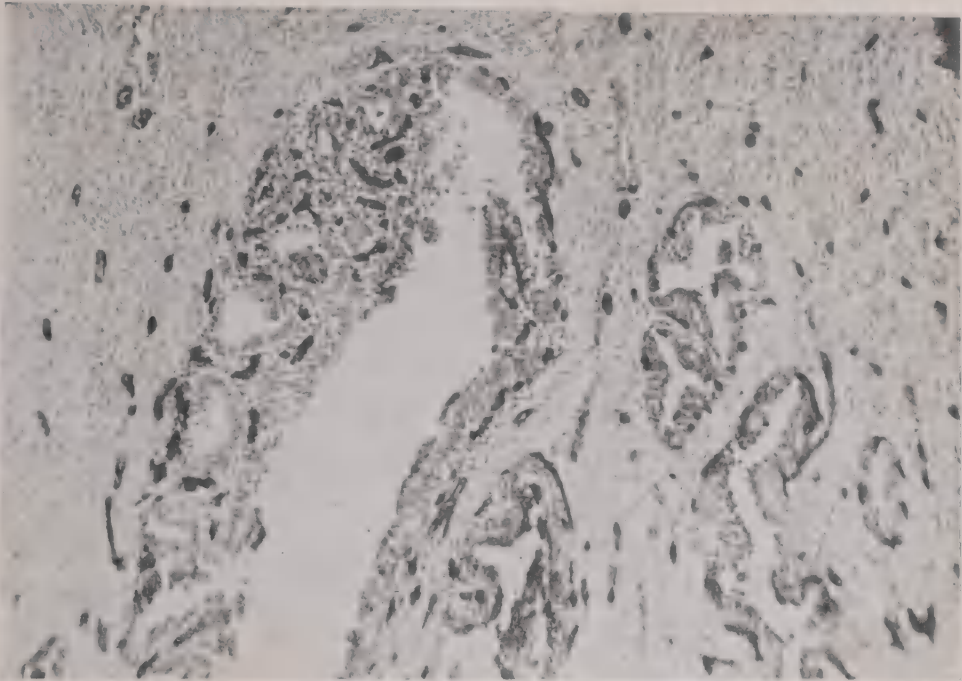
In 1935, Kutscher and Wolbergs, while studying the source of acid phosphatase in the urine of men, discovered that normal adult prostate tissue is rich in this enzyme. Fig. 92 shows serial sections of prostate gland, in one of which the alkaline phosphatase distribution is demonstrated, and in the other the acid phosphatase. The former is the normal phosphatase of bone and has an optimum pH of 9-9.5, while acid phosphatase acts best at pH 4.8. The high content of acid phosphatase in this tissue is quite apparent from this figure. The enzyme is characteristic of adult, but not immature, prostatic tissue, and it is found in large amounts in the urine of men. The daily output for a given man is fairly constant, but after the age of 50 years the amount excreted diminishes. Women and children excrete very little. The acid phosphatase content of the urine may therefore be considered an index of prostatic secretion, and hence a criterion of the functional state of the prostatic epithelium.

Primary tumors of the prostate are also rich in acid phosphatase, and the same is true of metastatic lesions of prostatic cancer occurring in bone. When such lesions are present, greatly increased concentrations of this enzyme in serum may occur, but this is not always the case. In other words, a high acid phosphatase of serum is indicative of metastases of prostatic cancer, but a low content is not necessarily a negative sign.

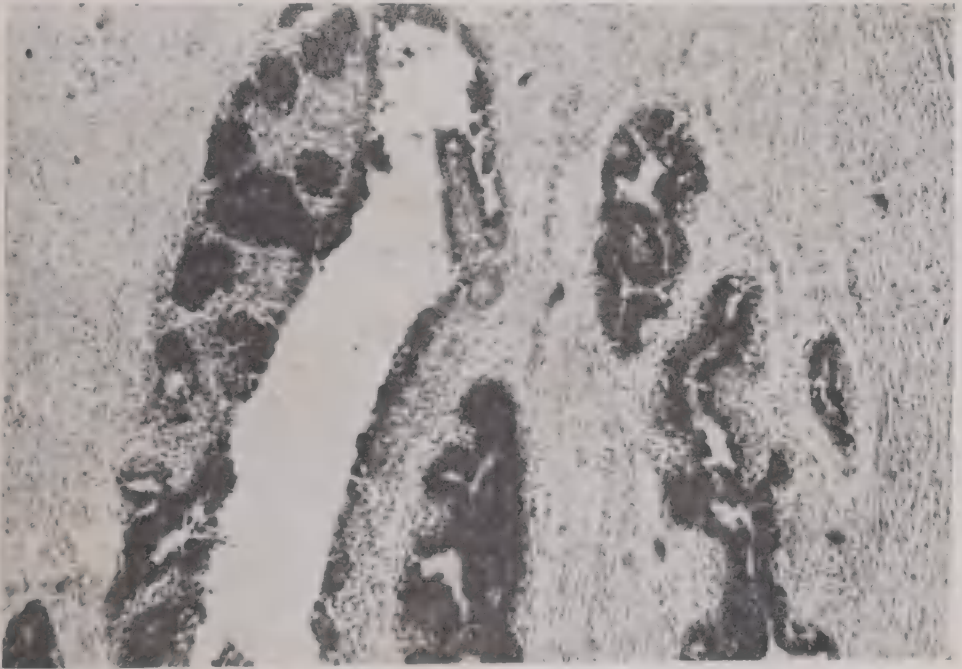
The treatment of prostatic tumors mentioned previously, i.e., castration or administration of estrogens, besides having favorable clinical effects, usually results in a prompt fall in the level of serum phosphatase. The administration of androgens, on the other hand, raises the acid phosphatase of the serum and results in untoward symptoms.

### PENICILLIN AND OTHER ANTIBIOTICS

In 1929 Alexander Fleming, Professor of Bacteriology at the University of London, noticed that staphylococcus colonies became transparent if they were in the immediate vicinity of a contaminating mold. It appeared that the staphylococci had undergone lysis, and he believed that this was due to some water-soluble substance produced by the mold. Since the mold was a penicillium, he named the unknown but effective substance "penicillin." He studied the properties of the substance, found that it was nontoxic to animals, even in enormous dosage, and suggested that it might be employed in human therapy



A.



B.

Fig. 92.—The distribution of alkaline and acid phosphatase in the adult human prostate. Serial sections stained for (A) alkaline phosphatase and (B) acid phosphatase.

A, Alkaline phosphatase. Only capillaries are positive; the glandular epithelium is negative. For this demonstration the section was incubated in a solution containing the substrate, sodium glycerophosphate, and calcium nitrate at pH 9.0. Alkaline phosphatase liberates phosphate ions which reacted with the calcium to give precipitated calcium phosphate at the site of the enzyme. The section was then exposed to a dilute solution of cobaltous nitrate, yielding cobalt phosphate, which is subsequently transformed into black cobalt sulfide by means of ammonium sulfide. The intensely dark spots, therefore, indicate the site of alkaline phosphatase.

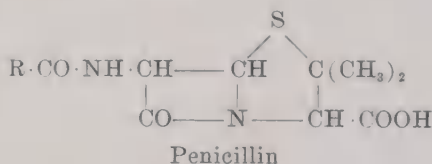
B, Acid phosphatase. The glandular epithelium is intensely positive; the capillaries are negative. The procedure is very similar to that used in A except that the incubating mixture contains lead instead of calcium and is buffered at pH 5.0. The resulting precipitate of lead phosphate is finally converted to black lead sulfide. (Courtesy Dr. George Gomori; see Gomori, G.: Proc. Soc. Exper. Biol. & Med. 42: 23, 1939; Arch. Path. 32: 189, 1941.)



as a bacteriostatic agent in areas infected with organisms sensitive to this agent. Fleming's observations received little attention until 1940, when they were re-examined by a group of investigators at Oxford, headed by Florey. They confirmed Fleming's work and extended it materially.

Penicillin has a very low toxicity to man and other mammals and can therefore be used therapeutically. It is very effective *in vitro* against most of the gram-positive cocci and bacilli and the gram-negative diplococci but is not bacteriostatic toward the gram-negative bacteria, the tubercle bacillus, malarial organisms, Friedländer's bacillus, and many others. Thus it is found to be a very powerful agent against streptococci, staphylococci, pneumococci, gonococci, meningococci, the clostridium group, spirochetes, and actinomycetes. Its activity against most of these organisms is at least one thousand times greater than that of the sulfonamides. Its action *in vivo* closely parallels its activity *in vitro*. A heavy initial dose is advised to prevent the development of resisting strains of the offending organism. This principle is applicable to antibiotics in general. An antibiotic is defined as a substance which is produced by a microorganism and is harmful to other microorganisms.

Penicillin is a complex monobasic organic acid, the structure of which has been studied by a large group of British and American scientists working in university and commercial laboratories. The probable structural formula is given below. There appear to be at least five forms of penicillin, each of which differs from the others in the group R.



These penicillins are produced simultaneously by the mold and have been designated by different letters in America and by Roman numerals in Britain. They are summarized in Table LI. The mold is capable of producing penicillins having different R groups if an appropriate precursor is added to the culture medium. About fifty active compounds have been formed in this manner.

Of these, G penicillin is the most important. G penicillin has been synthesized—still another triumph accomplished by the brilliant teamwork of American and British chemists (du Vigneaud). The free or acid penicillins are unstable, particularly in aqueous or alcoholic solution. The sodium and calcium salts, which have been studied more generally, are more stable in

TABLE LI  
THE PENICILLINS

NAME	R	NAME OF R
F Penicillin (Penicillin I)	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}=\text{CH} \cdot \text{CH}_2-$	$\Delta^2$ -Pentenyl
Dihydro F Penicillin	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2-$	n-Amyl
G Penicillin (Penicillin II)	$\text{C}_6\text{H}_5 \cdot \text{CH}_2-$	Benzyl
X Penicillin (Penicillin III)	$\text{C}_6\text{H}_4\text{OH} \cdot \text{CH}_2-$	<i>p</i> -Hydroxybenzyl
K Penicillin	$\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2-$	n-Heptyl

lution. They are crystalline compounds. The sodium salt is a light orange-brown, hygroscopic powder having a slight odor. It is quite soluble in water and alcohol but is inactivated by the latter. The calcium salt is less hygroscopic and for that reason is the form in which penicillin is employed when prepared in tablet form for oral administration and in a beeswax-peanut oil emulsion for slow absorption. In the dry state it is stable to light, but not to heat, and may be kept for months without losing its potency. When dissolved in water it tends to lose its activity but at low temperatures keeps well for several days. Two new types of penicillin have been developed which have the advantage of long-continued action. Thus one injection of procaine penicillin will have an effect over several days, and dibenzylethylenediamine dipenicillin will act for several weeks.

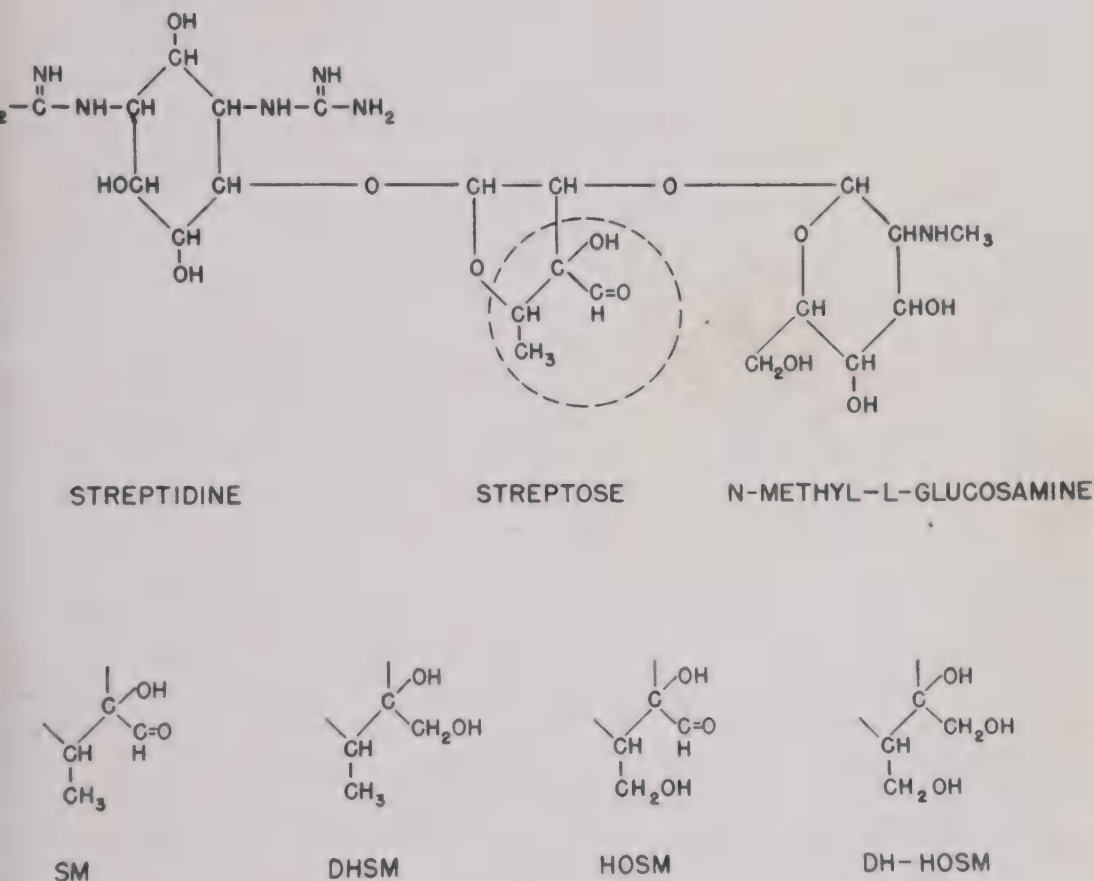


Fig. 93.—Chemical structure of streptomycin and derivatives. Abbreviations: SM = streptomycin; DHSM = dihydrostreptomycin; HOSM = hydroxystreptomycin; DH-HOSM = dihydrohydroxystreptomycin. (From Umbreit, W. W.: Tr. New York Acad. Sc. 15: 8, 1952.)

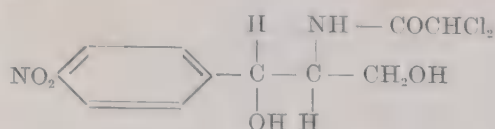
The production of penicillin involves (1) inoculation of a suitable sterile medium with the spores of a pure culture of *Penicillium chrysogenum*, (2) growth of the mold under careful control of air and temperature, (3) separation of the liquid medium containing the penicillin, and (4) separation, concentration, and purification of the penicillin. Penicillin may be administered intravenously, intramuscularly, intrathecally, or topically. If given orally about five times as much of this drug is required, due to destruction and non-

absorption in the gastrointestinal tract. It is rather rapidly excreted by the kidneys, probably through the renal tubules. (Rammelkamp.)

Streptomycin is second in importance only to penicillin. It was discovered by Waksman and associates in a deliberate search for an antibiotic which would be capable of exerting an inhibitory effect upon gram-negative bacteria. Streptomycin is produced by an organism, *Streptomyces griseus*, related both to bacteria and to molds. It is an organic base, soluble in water and insoluble in organic solvents, and can be obtained as crystalline salts. The hydrochloride or sulfate is commonly used. The empirical formula is  $C_{21}H_{39}N_7O_{12}$ . Upon hydrolysis a number of compounds results, and the nature of these reveals its structure. There is produced streptidine and streptobiosamine. Streptidine, 2,4,5,6-tetrahydroxy-1,3-diguanidocyclohexane, is attached through a glycosidic linkage to streptobiosamine, which is a nitrogen-containing disaccharide-like compound. The two sugars which comprise the latter are streptose,  $C_6H_{10}O_5$ , and N-methyl-L-glucosamine. Streptidine is shown in Fig. 93.

Streptomycin is much more stable than penicillin. Sterile solutions maintain their activity for two weeks or more at 37° C., and heating to 100° C. only partly inactivates them. It is somewhat more toxic than penicillin. Its great advantage is that it is effective in conditions which penicillin does not influence. Such are tularemia, tuberculosis, meningitis, and bacteremias due to Gram-negative bacteria, and certain enteric and urinary infections. (Pruess.) The introduction of two H atoms into the streptose portion, changing the aldehyde group to an alcohol, produces dihydrostreptomycin. This is just as potent as streptomycin. Of the two, streptomycin is thought to have a more deleterious effect upon the vestibular apparatus, and dihydrostreptomycin has its toxic effect upon the cochlear apparatus. A mixture of equal parts of each lessens these toxic actions.

There are many other antibiotics; i.e., substances produced by microorganisms which have bacteriostatic or bactericidal properties. Besides penicillin and streptomycin, several are now being used widely. Three are designated "broad spectrum" antibiotics; namely, chloramphenicol, Aureomycin, and Terramycin.\* The similarity in structure of Aureomycin and Terramycin is striking. Chloramphenicol is much simpler and was the first of the important antibiotics to be synthesized and manufactured by chemical methods. (Controulis.) All three are characterized by antimicrobial action against Gram-positive and Gram-negative bacteria, certain Rickettsiae, and large viruses. Chloramphenicol is also effective in typhoid fever. All three have low toxicity and may be administered orally.

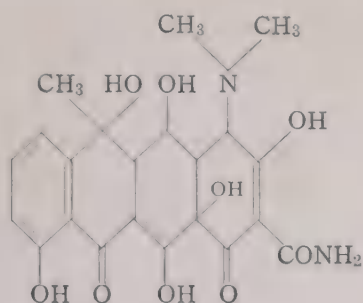


Chloramphenicol

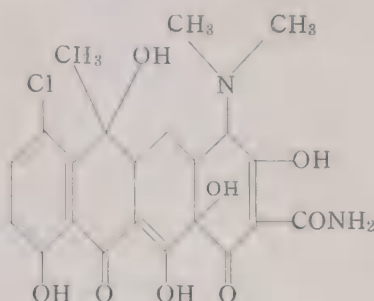
\*Terramycin and Aureomycin have been designated officially "oxytetracycline" and "chlortetracycline," respectively. Chloramphenicol is the official name for the antibiotic formerly called Chloromycetin.



Among the scores of other antibiotics isolated and investigated may be mentioned tyrothricin, bacitracin, polymyxin, neomycin, viomycin, and subtilin.



Terramycin



Aureomycin

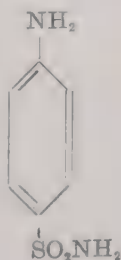
*B. brevis* produces tyrothricin, which contains the two mixtures of polypeptides, gramicidin and tyrocidine. They act chiefly upon Gram-positive organisms. Bacitracin, a polypeptide, is derived from *B. subtilis* and acts upon many organisms of the staphylococcal and streptococcal groups. (Johnson.)

## THE SULFA DRUGS

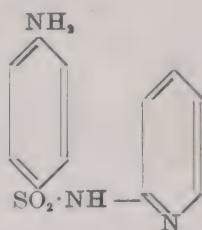
Although no exhaustive account of chemotherapeutic agents can be given in this work, some mention of a few of the more important developments will not be amiss. The most important advance of this nature since the discovery of arsphenamine—indeed, probably of even greater value—was the synthesis and application of the sulfonamide derivatives.

Sulfanilamide was first synthesized in 1908 by Gelmo and was widely used in the dye industry, but its antibacterial power was not suspected for many years. Complex derivatives, prontosil and neoprontosil, were shown in 1932 by German investigators to have a protective action against streptococcal infections in mice, and for a while both were used clinically, particularly neoprontosil. In the meantime French workers had postulated that these compounds were hydrolyzed in the body to form free sulfanilamide and that it was this compound which had the antibacterial activity. English and American scientists also took part in the studies. The early work of Long and Bliss in this country has been followed by many hundreds of investigations of both an experimental and a clinical character, and as a result the use of these drugs is on a sound basis today and many lives are constantly being saved because of their efficacy.

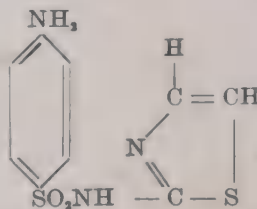
Sulfanilamide is para-aminobenzenesulfonamide.



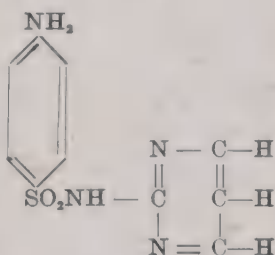
The ortho and meta compounds are quite inactive. All of the "sulfa" drugs are derivatives of sulfanilamide; those most commonly used, in addition to sulfanilamide, are:



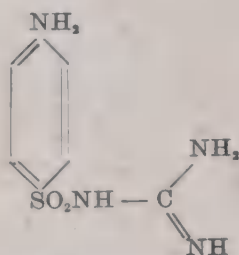
Sulfapyridine



Sulfathiazole



Sulfadiazine

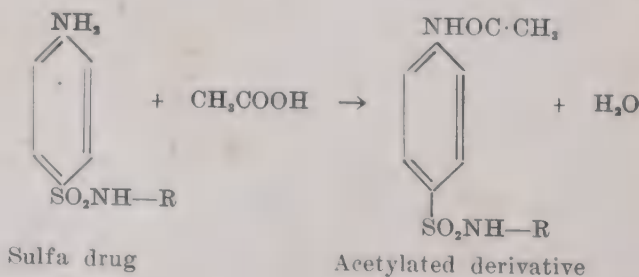


Sulfaguanidine

The relation of these substituted groups to compounds of biological importance may be pointed out. The thiazole group is also present in thiamine, as is the pyrimidine nucleus of sulfadiazine. Niacin and pyridoxine both have the pyridine ring, which is seen in sulfapyridine. Guanidine itself is a toxic substance, but creatine, a substituted guanidine, is a nontoxic physiological compound, and sulfaguanidine is no more toxic than the other sulfa drugs.

The five principal sulfa drugs are white crystalline compounds, only slightly soluble in water. They are not absorbed from the stomach to any appreciable extent, but sulfanilamide is absorbed throughout the remainder of the alimentary canal, as well as from serous cavities and open wounds. The others differ in the rapidity of absorption; this is largely related to their solubilities. The effective dosage depends both upon the rate of absorption and the rapidity of excretion.

As is the case with many other organic compounds, the sulfa drugs are changed during their passage through the body. An acetic acid group is introduced in the free amino radical. As a rule, about 10 to 20 per cent of the circulating drug is conjugated in this way. Coenzyme A is required for this acetylation.



Whether this acetylation results in lessened toxicity is not definitely known as yet, but the acetyl forms have no bacteriostatic action and, therefore, as the drugs

are rendered inert by acetylation, additional doses must be given. For effective medication frequent analyses of the blood must be made in order that the therapeutic level of the drug be kept constant. This depends not only upon the rapidity of absorption and excretion and the rate of acetylation, but also upon the degree to which these drugs are bound by the plasma proteins. When bound in this way they are believed to be inactive.

All five of these drugs are effective upon oral administration and diffuse into all tissues and body fluids to different degrees. They are eliminated chiefly by way of the kidneys, and some crystallize out too readily in the urinary passages (see Fig. 70). To avoid this, Lehr recommends the use of mixtures of different sulfonamides instead of a single one. Two or more of these compounds are soluble in one menstruum to the extent of their individual solubilities. Therefore, a half saturated solution of sulfadiazine may be half saturated with sulfathiazole and the resulting fluid will not be fully saturated and will not permit either compound to crystallize out. The sulfa drugs differ in the organisms which they attack, but in general it may be said that they are effective against streptococci, staphylococci, meningococci, and pneumococci and a number of other pathogenic organisms. They are bacteriostatic and to some extent bactericidal in vitro as well as in vivo. These compounds are "biochemical antagonists" to some essential cell component, as discussed in Chapter 24.

An interesting vitamin relationship is that which has been shown to exist between the sulfonamide drugs and some of the B vitamins; namely, folic acid, biotin, and inositol. The inclusion of one of the sulfa drugs in a synthetic diet fed to rats leads to deficiency symptoms which can be relieved by the addition of biotin, folic acid, and inositol. These factors can be synthesized ordinarily by the rat and consequently are not needed in the diet of that animal. Presumably their synthesis is effected by the intestinal flora. The sulfa drugs cause the vitamin deficiency by preventing the growth of the microorganisms. Folic acid appears to be specifically concerned in some way in maintaining the number of leucocytes and the proportion of granulocytes in rats. Feeding sulfaguanidine or Sulfasuxidine leads to leucopenia and granulocytopenia in these animals, and crystalline folic acid corrects the condition. Similarly the administration of sulfaguanidine or Sulfasuxidine may inhibit the formation of vitamin K by intestinal organisms, and a diminution of prothrombin may ensue.

Besides the five principal sulfa drugs mentioned, a large number of others have been synthesized. Many of them are in common use, and undoubtedly one will supplant one or more of these five. Sulfamerazine and Sulfamethazine are methyl derivatives of sulfadiazine; the former is a monomethyl and the second a dimethyl derivative, the methyl group being substituted for hydrogens on the pyrimidine ring. Both are more soluble than sulfadiazine and are used about the same way as that drug. Sulfasuxidine is succinyl sulfathiazole. This is one of the least toxic of this series and is being used to sterilize the intestinal tract. Sulfaguanidine is also recommended for this purpose. However, the results of the animal experiments described, in which sulfa drugs were used, should be kept in mind when treating human beings with intestinal anti-



septics. Sulfacetamide, an extremely soluble derivative, is used with great success in the treatment of ophthalmic infections.

All of the sulfonamides are somewhat toxic—some are more so than others—and some individuals are more susceptible than others. Therefore they should always be used with the utmost caution.

## RADIOACTIVE ISOTOPES

Several radioactive isotopes have been the subject of biological and clinical investigation as a result of the recent intense activity in this field. Brief mention may be made of two: radioactive iodine and radioactive phosphorus.

Radioactive iodine may be used as a diagnostic test for hyperthyroidism. One hundred microcuries of  $I^{131}$  in distilled water is taken orally and the absorption of this by the thyroid gland may be measured by placing a Geiger-Müller tube over the gland either twenty-four or forty-eight hours after swallowing it. The thyroid of the average normal person retains less than 30 per cent, that of a patient suffering from myxedema concentrates still less, while an uptake of over 35 per cent places the individual in the definitely "toxic" range. Usually the greater the uptake, the more toxic is the patient clinically. This method is said to be more accurate than the determination of either the B.M.R. or the protein-bound iodine of the blood. (Hamilton; Jaffe.) Another procedure is the determination of protein-bound radioiodine (PBI<sup>131</sup>) after administration of tracer doses. (Friedberg; Silver.) McConahey has shown that, three days after administration of  $I^{131}$ , the level of PBI<sup>131</sup> was highest in hyperthyroid patients, virtually absent in myxedema cases, and in between in persons with no thyroid abnormality.

In larger doses the same isotope is used to destroy thyroid tissue. It is said to be better than the use of roentgen rays and is the method of choice in certain types of toxic goiter. (Gordon and Albright.) The dosage may be gauged from the percentage uptake of a radioiodine tracer dose and the estimated weight of the thyroid gland. (Rawson and Rall.) Radioactive phosphorus is also used therapeutically. It is found to give good results in the treatment of refractory cases of polycythemia vera and is a helpful adjunct in the management of chronic leucemia. (Erf and Lawrence; Duffy and Howland.)  $P^{32}$  is taken up first by the erythrocytes, later by the leucocytes. It also lodges, to a lesser extent, in rapidly growing tumors, and hence is used in tracer studies in cancer diagnosis.

## ENZYMES AND ENZYME INHIBITORS

**Mucolytic Enzymes.**—Mucolytic enzymes occur both in microorganisms and in animal tissues and fluids. They catalyze the hydrolysis of highly polymerized mucopolysaccharides. These complex carbohydrates likewise are found both in microorganisms and in animal tissues and are built up from hexosamines and uronic acids. Lysozyme and hyaluronidase are examples of mucolytic enzymes which act upon these carbohydrates. Avidin and bacteriophages may also prove to belong to this group.

Lysozyme was discovered by Fleming. It is a globulin and occurs in egg white, tears, saliva, nasal secretions, and leucocytes. It is also found in some microorganisms. It has a bactericidal action upon some microorganisms as a result of its attack upon the mucopolysaccharides present in the microbes. The products of the reaction are the simpler carbohydrates mentioned. Under the influence of lysozyme the microorganisms swell to several times their normal size, the Gram stain becomes negative, and nonprotein nitrogenous substances, inorganic phosphates, and the simple carbohydrates go into the surrounding medium. The enzyme is a basic protein having a molecular weight of 18,000 and is unlike most other enzymes in being heat- and acid-resistant. It is, however, unstable toward oxidation and alkali. It has been crystallized. (Alderton.)

The functions of lysozyme are a subject for speculation. It is possible that it has a protective action when tissues are in a weakened condition. Thus, in vitamin-A deficient rats the xerophthalmia could be relieved by washing the eyes with human tears. (Findlay.) And in xerophthalmia in human beings a low concentration of lysozyme was found. Treatment with vitamin A raised it and, simultaneously, the condition was improved. (Andersen.) The occurrence of lysozyme and similar enzymes in some microorganisms is explained by Meyer and co-workers by the assumption that these enzymes are involved in some metabolic process connected with the carbohydrate substrates in the membranes of these microbes. These membranes are sometimes very tough, and it is also possible that the lysozyme softens them up preliminary to cell division.

Large amounts of lysozyme seem to be harmful. Meyer and his group have recently linked lysozyme activity with ulcerative conditions of the gastrointestinal canal. In the first place, the enzyme is present in gastric mucosa and in the gastric juice of man. When ulcers are present, it is found to be in higher concentration, and when the ulcers are under control, the lysozyme is diminished, probably because it has been digested by pepsin. Similarly the stools of patients suffering from chronic ulcerative colitis were found to have a lysozyme content twenty-seven times that of normal stools. An ulcer could be produced experimentally in a Pavlov pouch dog by the instillation of crystalline lysozyme. It was felt that lysozyme acted upon surface mucus, digesting it away. Then it was presumed that the pepsin and HCl acted upon the unprotected mucosal tissue, eroding it to produce an ulcer. However, grave doubt has been cast on an etiological role for lysozyme. (Glass; Gray.) Nevertheless, this enzyme appears to be an index of the severity of the disease process and a measure of functioning colonic tissue.

Lysozyme and avidin (see page 298) have similar properties and similar distribution and for a while it was thought that they might be identical. However, several differences have been brought out. For example, highly purified lysozyme has negligible avidin activity while retaining its power to hydrolyze the polysaccharide. It may also be stated that lysozyme and bacteriophage are not identical, although it is probable that the two are associated in bactericidal action.

Hyaluronidase is the enzyme which catalyzes the depolymerization of hyaluronic acid. This mucopolysaccharide was first isolated from a type II pneu-



nococcus and has since been found in the vitreous humor, the umbilical cord, the synovial fluid, and in certain tumors. (Meyer.) However, its most significant location is the skin, which probably contains the largest store of this substance. Hyaluronic acid is a polymer of acetylglucosamine and glucuronic acid, with a molecular weight of 200,000 to 400,000 or even higher, when present in some semisolid gels. It seems to bind water in the interstitial spaces, forming a sort of jelly, which holds the cells together. The enzyme is believed to be identical with the "spreading factor." This is an agent which facilitates the diffusion of substances into tissues. If a suitable indicator, such as hemoglobin or India ink, is injected intradermally, it will, under normal conditions, remain localized. If the spreading factor is present, the indicator will diffuse over a wide area, depending upon the amount of the factor present. The hyaluronic acid present in the intercellular substance of the dermis apparently offers resistance to the spread of large aggregates, and when it is disintegrated by the enzyme (spreading factor) the resistance is abolished or at least diminished. Hyaluronidase is found in many microorganisms, as well as in extracts of leech heads and in snake and bee venoms. It also is present in spermatozoa; in fact, the most convenient source for its preparation is bull or ram testes.

Hyaluronidase may have a very important bearing on the spread of disease germs in the body. Since many microorganisms produce this enzyme, it has been suggested that it determines their invasiveness. The hyaluronic acid present in the tissues acts as a physiological barrier to the organism, but as the organism produces the enzyme, which disintegrates the barrier, it spreads with ease into the surrounding tissues. It is believed that the efficacy of salicylates in rheumatic fever is due to a specific inhibition of hyaluronidase, thus preventing the breakdown of hyaluronic acid.

Highly purified hyaluronidase is finding practical use clinically in facilitating hypodermoclysis; i.e., the administration of large volumes of fluids hypodermically. It is given prior to, or simultaneously with, the fluid and hastens its flow and absorption remarkably. It also has been used to enhance the penetration of drugs, such as penicillin, into mucous membranes and to spread the effect of local anesthetics over a wider area.

**Enzymatic Débridement.**—On page 194 it was stated that fibrin could be digested by an enzyme, "fibrinolysin" or "plasmin," and that a precursor of this enzyme, present in human plasma, could be activated by streptokinase. This activator is derived from certain streptococci. It has been purified and, in conjunction with the proenzyme, profibrinolysin or plasminogen, forms a powerful system for the rapid lysis of human fibrin. There is also produced by streptococci an enzyme which digests desoxyribonucleoprotein. This nucleoprotein and its nucleic acid constitute from 30 to 70 per cent of thick, purulent exudates. The enzyme has been named "streptodornase." Since the material present in many infected or necrotic wounds, burns, or other lesions contains both fibrinous and purulent matter, the two enzymes are administered in combination to effect solution of the fibrin and nucleoprotein. The agent is known as "streptokinase-streptodornase" and is being used clinically. It is essential that the enzymes remain in close contact with the area to be treated



for several hours. They have no antiseptic or antibiotic action and do not digest mucin, collagen, or fibrous tissue. (Tillett; Christensen and MacLeod; Sherry and Goeller.)

**Enzyme Inhibitors.**—The synthesis, by Roblin and Clapp, of heterocyclic sulfonamides possessing marked carbonic anhydrase-inhibiting powers has led to a number of investigations which may have clinical application. One of these compounds, named Diamox, has been found to cause diuresis when given intravenously, or even orally, to patients with congestive heart failure. (Friedberg.) This effect is attributed to an inhibition of the formation of  $H^+$  ions in the renal tubule cells, permitting the greater secretion of  $Na^+$  and water. The same inhibitor is effective in diminishing the secretion of gastric  $HCl$ , and also the volume output and bicarbonate content of pancreatic juice. (Janowitz and Hollander; Hollander and Birnbaum.) The possible use of such an agent in controlling peptic ulcers is apparent.

### CORTISONE AND ACTH IN RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic disease affecting the joints, characterized by pain, deformity, limitation of motion, and sometimes bony ankylosis, and resulting in debility and weakness. In the past the most useful therapeutic agents have been salicylates, combined with rest, improved nutrition, general hygiene, and, if possible, a warm dry climate. Evaluation of new drugs has been difficult, because remissions frequently occur spontaneously for varying lengths of time. However, the discovery by the Mayo group, in 1949, of the effect of cortisone and ACTH was dramatic and conclusive.

Twenty years previously, Hench had made the observation that if patients suffering from rheumatoid arthritis became pregnant or jaundiced, there was a prompt relief of the arthritic symptoms. On this basis he tested many bile and hormonal derivatives for their value in this condition without avail, until finally Kendall's compound E (see page 608) became available. This is 11-dehydro-17-hydroxycorticosterone and is called "cortisone." It effects a decrease in the stiffness, tenderness, and pain in the joints in a few hours or days. Diminution of the joint swellings, disappearance of soft tissue deformities, and general improvement in health occur more slowly. ACTH, which, of course, stimulates the production of cortisone, has an effect which parallels that of cortisone in every particular. Cortisone is now produced on a large scale by "partial synthesis" (Sarett) and is being used not only for rheumatoid arthritis, but also for a number of other ailments, including acute rheumatic fever, acute asthma, and Addison's disease.

The mechanism whereby cortisone produces its effect is not known. Apparently it is replacement therapy, since it must be administered continually, and, obviously, ACTH functions by stimulating the adrenal cortex to produce this as well as other adrenal cortical hormones. Sayers suggests the following possible theories to account for cortisone's action: (1) interference with the release of some anaphylactogenic substance or with its toxic action, (2) alteration in cell permeability as a result of the influence of the hormone upon hyaluronidase, or (3) the suppression of responses in mesenchymal tis-

sues. In connection with (2), Seifter and associates have shown that adrenal cortical extract can abolish the enhancing effect of hyaluronidase on permeability of tissue membranes.

The explanation offered for the temporary suppression of arthritis during pregnancy and jaundice is that in these conditions the anterior pituitary is stimulated to increase its output of ACTH, which in turn causes the adrenal cortex to produce more cortisone, and the cortisone produces the beneficial effect against arthritis. In this connection it is interesting to note that Grainger was able to induce remission of rheumatoid arthritis by the intravenous administration of suitable amounts of post-partum plasma.

As might be expected, cortisone sometimes has unpleasant side effects. Some of these remind one of the adrenogenital syndrome (page 612). Others are hypertension, headaches, skin eruptions, confused mental states, and even diabetes, which may disappear when the cortisone treatment is discontinued. As a result, there are quite a number of contraindications to its use.

### PYROGENS

Parenteral administration of nutrients or medicinal substances dissolved in sterile distilled water sometimes gives rise to febrile reactions which have been attributed to "pyrogens." These pyrogens are metabolic products of microorganisms which are present in the medicinal, in the water employed as the diluent, or which result from trace microbial growth, occurring subsequent to the preparation of the parenteral solution and prior to its sterilization. Pyrogens are relatively heat stable, although syringes and needles may be rendered pyrogen-free by heating at 250° C. Such factors as the nature of the pyrogen, temperature of heating, pH, time of heating, and interaction with medicinals present serve to influence the rate of thermal destruction. Consequently, heating is usually of no value in the case of biologicals. Oxidizing agents, such as permanganate and peroxide, are known to destroy pyrogens but are impractical for use with most medicaments. Absorptive agents, e.g., charcoal, kaolin, and alumina, are moderately effective for removal of pyrogens. Filtration through conventional bacteriologic filters is relatively ineffective, but filtration through special compressed asbestos pads of extremely low porosity has been claimed to be a reliable depyrogenizing procedure for limited volumes of "parenterals." In general the most practical solution to the problem is to avoid contamination with pyrogen-producing organisms. Such organisms are present almost everywhere, and the utmost care and cleanliness are required in the equipment employed and the technique used in the preparation of parenterals to prevent their growth. Commercial firms routinely subject their parenteral products to the rabbit pyrogenicity test described in the U. S. Pharmacopeia.

The chemical composition of pyrogens has not been fully elucidated. In fact the essential question of whether pyrogens from various microbial sources are the same or different has not been unequivocally answered, although they are probably different. The limited data available would describe pyrogens as relatively stable polysaccharide complexes, containing significant amounts



of nitrogen. At least a portion of the nitrogen is attributable to amino-sugars. Evidence for the presence of bound lipids and a phosphorus containing moiety exists. Nucleic acids have been reported to be frequently associated with pyrogens and to be extremely difficult to remove. It is generally agreed that pyrogens are not proteins.

Fever therapy in some form has long been used in the treatment of disease. So-called nonspecific, foreign protein therapy dates back to the eighteenth century, but it was only recently recognized that the vague phenomena included under this heading had a common etiology; namely, pyrogens. Trace microbial contamination of a protein is usually sufficient to result in the formation of enough of the polysaccharide complex to impart pyrogenic activity. Several relatively pure pyrogenic polysaccharide complexes have been described recently. Their availability has stimulated investigations, leading to the realization that these substances, which are active even in microgram quantities, are capable of eliciting a miscellany of complex physiological changes. These effects, produced even by subfebrile doses, apparently involve the reticuloendothelial and pituitary-adrenal systems.

One of the most constant effects in animals and man is a rise in the number of circulating leucocytes, preceded by a brief diminution. Histological studies after administration of a purified pyrogen from a *Pseudomonas* species have revealed stimulation of bone marrow and lymphoid tissues, together with changes in the adrenals, and, to a lesser extent, in certain of the other endocrine organs. Corticosteroids are secreted, while the adrenal ascorbic acid is simultaneously decreased. In this sense the response resembles that produced by ACTH. There is also evidence that trophic hormones other than ACTH are also secreted. It appears that some of the effects may be exerted through the pituitary-adrenal system, while others may be the result of direct action on other tissues. The latter is indicated by the fact that purified bacterial polysaccharides do affect the metabolism of leucocytes in tissue culture.

It is impossible as yet to predict precisely what role pyrogens will play in therapeutics, but their isolation in purified form and the accumulation of fundamental biochemical and physiological information have established a rationale for their use. Current clinical research indicates that these pyrogenic polysaccharide complexes may be of real value in allergies, dermatoses, and ophthalmologic conditions. They have also shown some promise in certain neurological disorders. These pyrogens should be distinguished from the one produced in inflammations which is of a protein nature (see page 663).

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## Appendix

### Standard Buffer Solutions

*Clark and Lubs standards* are prepared by adding to 50 c.c. of 0.2 M potassium chloride the indicated number of cubic centimeters of 0.2 N hydrochloric acid and diluting to 100 c.c.

pH	1.2	1.4	1.6	1.8	2.0	2.2
c.c. HCl	64.5	41.5	26.3	16.6	10.6	6.7

The next series is prepared by adding to 50 c.c. of 0.2 M acid potassium phthalate the indicated number of cubic centimeters of 0.2 N hydrochloric acid and diluting to 200 c.c.

pH	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8
c.c. HCl	39.60	32.95	26.42	20.32	14.70	9.90	5.97	2.63

Another series is prepared by adding to 50 c.c. of 0.2 M boric acid in 0.2 M potassium chloride the indicated number of cubic centimeters of 0.2 N sodium hydroxide and diluting to 100 c.c.

pH	8.2	8.4	8.6	8.8	9.0	9.2	9.4	9.6	9.8	10.0
c.c. NaOH	5.9	8.5	12.0	16.3	21.3	26.7	32.0	36.85	40.8	43.9

*Walpole acetate standards* are prepared by mixing 0.1 N solutions of acetic acid and sodium acetate in the following proportions:

pH	c.c. 0.1 N Acetic acid	c.c. 0.1 N Sodium acetate
3.6	185	15
3.8	176	24
4.0	164	36
4.2	147	53
4.4	126	74
4.6	102	98
4.8	80	120
5.0	59	141
5.2	42	158
5.4	29	171
5.6	19	181

*Sörensen phosphate standards* are prepared by mixing M/15 solutions of disodium phosphate and potassium acid phosphate in the following proportions:

pH	c.c. M/15 Disodium phosphate	c.c. M/15 Potassium acid phosphate
5.4	3.0	97.0
5.6	5.0	95.0
5.8	7.8	92.2
6.0	12.0	88.0
6.2	18.5	81.5
6.4	26.5	73.5
6.6	37.5	62.5
6.8	50.0	50.0
7.0	61.1	38.9
7.2	71.5	28.5
7.4	80.4	19.6
7.6	86.8	13.2
7.8	91.4	8.6
8.0	94.5	5.5



	7	24	49	19	*	440								6	7
1 piece (2X2X1 in.)															
7. Sponge 3 <sup>3</sup> / <sub>4</sub> pieces (1½X1½X2 in.)	8	5	54	32	*	293									

CEREALS

	13	2	70	10	3	357	0.051	0.400	0.0048	71	400-500	120	140 mg. choline 1.5-2.5 mg. niacin 1 mg. pantothenic acid	1
1. Barley, entire <sup>3</sup> / <sub>4</sub> c. scant														
2. Corn flakes 3½ c.	8	1	80	9	1	359	0.020	0.283	0.0029		+			2
3. Corn meal, yellow ¾ c.	8	1	78	12	1	356	0.016	0.152	0.0009	500-600	375-400	50-300	42 mg. choline 0.6-1.6 mg. niacin 0.8 mg. pantothenic acid 400 vitamin B₆ curative units	3
4. Farina, raw ⅝ c.	12	1	76	11	*	359	0.021	0.125	0.0008		70-125			4
5. Hominy, cooked or canned ½ c. scant	2	*	15	83	*	69	0.002	0.015	0.0002					5
6. Macaroni, cooked, plain ½ c.	4	*	19	75	*	96								6
7. Noodles (egg) dry ⅞ c.	14	5	71	9	*	385	0.022	0.144	0.0012					7
8. Oatmeal or rolled oats, raw 1¼ c.	14	7	68	8	1	396	0.069	0.392	0.0038	0-25	400-900	85-180	0.25 mg. B₆ 150 mg. choline 1.1 mg. niacin 1.3 mg. pantothenic acid	8
9. Oatmeal, cooked ½ c. scant	2	1	11	85	*	62	0.010	0.07	0.0013	0-3	58-130	12-15	50 vitamin B₆ curative units	9
10. Rice, brown, raw ⅝ c.	8	2	78	12	1	356	0.065	0.336	0.0020	50-100	200-525	150		10
11. Rice flakes 3 c.	8	*	82	8	1	363								11
12. Rice, puffed 6½ c.	7	*	83	9	*	363								12

Cereals proper contain no vitamin C. Their growing sprouts, however,  
are rich sources of this vitamin.

Selected from Table I XVIII of Howley, F. F. and Carden, G.: The Art and Science of Nutrition, ed. 2. St. Louis, 1944. The C. V. Mosby Co. by courtesy of the authors.

\*Data given are expressed as the nearest whole number for average composition of protein, fat, carbohydrate, water, fiber, and calories.

Data given are expressed as the nearest whole number for average composition of protein, fat, carbohydrate, mineral, and energy. Where no value is available for a food constituent the space is left blank. This does not mean that the food is inert in this particular substance, but only that no value has yet been found.

If a food constituent is known to be entirely absent, the fact is indicated by a zero, where no value is available for a food constituent the space is left blank. This does

If a value is less than 1%, the asterisk (\*) is used to indicate it.

Carbohydrate values quoted are total carbohydrate (by difference) exclusive of fiber which is indicated in another column.

Carbonyluric values quoted are total carbonylurate (by unmetenol) exclusive of urea which is indicated in enclosed column. Vitamin values are expressed in a weight basis (micrograms or gamma) for thiamine and riboflavin; as milligrams for ascorbic acid and niacin. Vitamins A and D are expressed as International units. Vitamin B<sub>6</sub> or pyridoxine is expressed as curative units. Where the vitamin is known to exist, but no numerical value has been determined, the fact is indicated by the plus sign.

The data used in compilation of this table were obtained from the sources indicated in the list at the end of the table.





3. Graham 12 large	8	10	74	6	1	419	0.020	0.203	0.0019					Cereal products do not contain ascorbic acid	3
4. Oyster 2 $\frac{2}{3}$ c.	10	10	73	6	"	416	0.022	0.102	0.0020						4
5. Saltines 15 double	9	12	71	5	"	427	0.025	0.100	0.0015						5
6. Soda, plain (N. B. C.) 15	10	10	73	6	"	416	0.022	0.102	0.0020						6
7. Sweet tea biscuit 20 $\pm$ according to kind	8	11	76	5	"	432									7
8. Whole wheat wafer 20	9	7	74	7	1	399									8

## DAIRY PRODUCTS

1. Butter 10 pats (1×1×½ in.)	1	81	•	16	0	733	0.015	0.017	0.0002	2,500- 5,000	100	0	40-150 I. U. vitamin D 200 curative units vitamin B <sub>6</sub>	1
2. Cheese, American Cheddar 1 slice (4½×1½×1¼ in.) ⅞ c. grated.	24	32	2	39	0	393	0.931	0.683	0.0013	2,000- 4,000	40-50	0	350 curative units vitamin B <sub>6</sub> 15-19 mg. choline 0.2 mg. niacin Mineral values given are for "hard cheese"	2
3. Cheese, cottage, skim milk ½ c. scant (6 tbs.)	19	1	4	74	0	101	0.124	0.177	0.0003	60-110	+	0		3
4. Cheese, Cream 1⅓ pkg.	7	37	2.3	53	0	368	0.06	0.080		2,000	15-20		0.012 mg. biotin 0.6 mg. niacin 1.40 mg. pantothenic acid	4
5. Cheese, Limburger ½ c. scant (6-7 tbs.)	24	32	1	38	0	388				1,400	+	0	0.4 mg. biotin 1.4 mg. niacin 5.8 mg. pantothenic acid	5
6. Cheese, Parmesan 1 slice (1×1×5 in.)	35	27	0	29	0	385	1.35	1.0	0.002	1,200- 1,500	22-30	0		6
7. Cheese, Roquefort 1 slice (1½×1¼×3½ in.)	22	33	1	37	0	376	0.75	0.683	0.002	4,000	+	0	0.08 mg. biotin 12.4 mg. niacin 9.6 mg. pantothenic acid	7
8. Cheese, Swiss 1 slice (4½×3½×1½ in.)	29	31	2	34	0	404	1.086	0.812	0.002	2,300	30	0	0.01 mg. biotin 0.7 mg. niacin 2.6 mg. pantothenic acid	8
9. Cream, 20%, "Coffee" 2/5 c.	3	20	4	73	0	210	0.10	0.09	0.0002	1,000- 1,500	30-40	1±	13 I. U. vitamin D	9
10. Cream, 40%, "Whipping" 2/5 c.	2	40	3	59	0	384	0.086	0.067	0.0002	2,000- 2,500	25-35	1±	27 I. U. vitamin D	10

TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL

EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 OZ.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIE (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (i. u.)	THIAMIN (μg)	RIBOFLAVIN (μg)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.	
DAIRY PRODUCTS—Continued																
11. Milk, acidophilus 2/5 c.	3	4	4							+	+	+			11	
12. Milk, buttermilk, churned from cream 2/5 c.	4	1	5	91	0	45	0 122	0 097	0 0003	+	6	180		0.35-0.56 mg. pantothenic acid	12	
13. Milk, buttermilk, cultured, skim 2/5 c.	4	*	5	91	0	36	0 105	0 097	0 0003	42	15-50	80			13	
14. Milk, condensed, sweetened 1/4 c.	8	8	55	27	0	327	0 370	0 235	0 0006	300-700	57	300	0		14	
15. Milk, evaporated, unsweetened 6 2/3 tbs.	7	8	10	74	0	139	0 250	0 200	0 0004	300-700	50-80	300-330	0	Irradiated, evaporated, 135 I. U. vitamin D per pint can 0.2 mg. niacin	15	
16. Milk, malted, dry 3/4 c.	15	9	71	3	0	418	0 357	0 345	0 0021	4,500	300-600	500-750		79 I. U. vitamin D	16	
17. Milk, powdered, skim 3/4 c.	36	1	52	4	0	359	1 220	0 960	0 0030		375	+++	0	10.5 mg. niacin 56 curative units vitamin B6 159 mg. choline	17	
18. Milk, powdered, whole 3/4 c.	26	27	38	4		496	0 900	0 696	0 0017	1,300-1,800	315	1,300-1,900	0	63 I. U. vitamin D 107 mg. choline	18	
19. Milk, skim, fresh 2/5 c.	4	*	5	91	0	36	0 122	0 096	0 0002	12	30-75	180	2 (raw) 1 (past.)	14 curative units of vitamin B6 0.2-0.4 mg. pantothenic acid	19	
20. Milk, whey 2/5 c.	1	*	5	93	0	27	0 044	0 035						0.2-0.6 mg. pantothenic acid	20	
21. Milk, whole, fresh 2/5 c.	4	4	5	87	0	69	0 120	0 093	0 0002	160-225	30-75	250-300	2 (raw) 1 (past.)	Irradiated 13.5 I. U. vitamin D Fortified 40 I. U. vitamin D 90 curative units vitamin B6 0.1-0.5 mg. pantothenic acid 14.7 mg. choline 0.1 mg. niacin	21	
FATS																
1. Cod-liver oil 7 tbs. (1 tbs. = 15 Gm.)	0	100	0	0	0	900				80,000-300,000 A and 8,000-30,000 D, i. e., 10:1					U. S. P. Standard 850 units vitamin A and 85 vitamin D units per Gm.	1
2. Corn, cottonseed, olive, and peanut oils 9 tbs.	0	100	0	0	0	900				0				Corn, 2,000 vitamin B6 curative units Peanut 5,000 vitamin B6 curative units	2	



FISH++

3. Lard, refined 62½ lbs.	0	100	0	0	0	0	900		0	3
4. O'conarcine 7¾ lbs.	1	81	•	16	0	733	0 02	0 0002	?	4
5. Suet, beef	2	93	0	5	0	844				5

[illegible]

†Fish is also valuable for its iodine content.

Fish (on the average) is estimated by Sherman to contain 0.109 Gm. calcium, 1.148 Gm. phosphorus, and 0.0055 Gm. iron per 100 Gm. of protein. On this basis, for each 20 Gm. of protein (the average listed here), the values would be 0.0212 Gm. calcium, 0.2296 Gm. phosphorus, and 0.0011 Gm. iron. When values do not appear, the average may be used or the value calculated on this basis for the protein content given.

TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL  
EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 OZ.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (I. U.)	THIAMIN (μg)	RIBOFLAVIN (μg)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
Fish—Continued															
15. Salmon, fresh 1 (3×4×¾ in.)	22	13	0	65		203	0.013	0.242	0.001	20-750	110	225-275		400-800 I. U. vitamin D 6 mg. biotin 6-8 mg. niacin	15
16. Sardine 11 (3 in. long)	21	25	1	50		313	0.035	0.365	0.0018	28,000	30-90	200			16
17. Scallops ½ c. (10-15)	15	0	3	80		74	0.117	0.040	0.003		+				17
18. Shad, fresh 1 (¾ in. cross section from back)	19	10	0	71		163	0.02	0.20	0.001			200			18
19. Shad roe ½ medium	21	4	0	71		118	0.023	0.242	0.0012	2,000- 4,200	200-300				19
20. Shrimp, drained, solids ¾-1 c.	26	1	0	78		112	0.094	0.172	0.0014		90				20
21. Sole, raw 1 (4×4×¾ in.)	17	1	0	80		76									21
22. Trout, brook 1 (2½×1½×2½ in.)	19	2	0	78		96									22
23. Trout, lake 1 (2½×1½×2½ in.)	18	10	0	71		170	0.018	0.202	0.001		87	200			23
24. Tuna, canned, flaked ¾ c.	24	11	0	63		194	0.034	0.290	0.0014	200		200			24
25. Whitefish, Great Lakes 1 (2½×1½×2½ in.)	23	7	0	70		150	0.018	0.202	0.001						25

FLOURS

1. Corn meal, yellow, bolted, degerminated ¾ c.	8	1	78	12	1	356	0.018	0.191	0.0009	350- 1,000	50-300	80-100		400 vitamin B <sub>6</sub> curative units 10 mg. choline	1
2. Rye, medium ¾ c.	7	1	79	11	2	358	0.018	0.289	0.0013	+	165-220	60-105		1.2 mg. niacin 1.3 mg. pantothenic acid	2
3. Soybean, high fat 1½ c. scant	37	20	12	7	3	379				200-500	600- 1,200	600		4.8 mg. niacin 0.8-2.2 mg. pantothenic acid	3
4. Wheat, graham, all types ¾ c.	13	2	72	11	2	360	0.039	0.364	0.0037	+	330-500	100-200			4

Current products contain  
no vitamin C

The minute quantities of vitamin C, originally present in fish muscle, are  
lost before consumption of the fish; therefore, fish may be  
considered as devoid of this vitamin

5. Wheat, patent, all purpose  
¾ c.

11	1	76	12	*	355	0.015	0.101	0.0010	0	60-100	40	Cereal products contain no vitamin B <sub>6</sub>	10.2 mg. B <sub>6</sub> , 52 mg. choline 1 mg. niacin 0.6 mg. pantothenic acid	5
12	2	74	9	2	368	0.035	0.306	0.0035	0-20	225-750	100-200	0.5 mg. B <sub>6</sub> , 6 mg. niacin 1.3 mg. pantothenic acid		6

## FRUITS

1. Apple 1 small	*	*	14	84	1	64	0.007	0.012	0.0004	40-100	20-50	73	2-8	25 vitamin B <sub>6</sub> curative units	1
2. Apricots, dry 10-15 halves	5	1	60	29	2	268	0.065	0.120	0.0076	5,000- 15,000	60-170	105-300	2-12		2
3. Apricots, fresh 2 avg. size	1	*	12	85	*	57	0.013	0.024	0.0006	3,000- 8,000	25-60	50-100	1-6		3
4. Avocado ½ of 4 in. fruit	2	7-26	3-6	65-82	2	105-265	0.045	0.044	0.0063	150-700	100-200	90-150	2-8	0.9-1 mg. niacin	4
5. Banana 1 small	1	*	22	75	*	99	0.008	0.028	0.0006	250	50-100	40-84	8-12	66 vitamin B <sub>6</sub> curative units 70 mg. pantothenic acid 0.4-0.6 mg. niacin	5
6. Blackberries 1 c.	1	1	8	85	4	62	0.017	0.019	0.0009	80-300	25		3		6
7. Blueberries ⅔ c.	1	1	14	83	1	68	0.025	0.020	0.0009	20-80	45	15	4-10		7
8. Cherries, sweet ⅔ c.	1	1	17	80	*	80	0.017	0.022	0.0008	15-550	51	180	8-15	0.1 mg. niacin	8
9. Cranberries 1 c.	*	1	10	87	1	53	0.013	0.011	0.0004	10-20		+	10-15	1.3 mg. niacin	9
10. Currants, fresh ½ c.	2	*	10	85	3	61	0.026	0.038	0.0007	120-400	30-45	+	Black 150-300 Red 40		10
11. Dates, dry 14	2	3	78°	15		347	0.070	0.056	0.0036	60-300	60-100	45	0	2.2 mg. niacin	11
12. Figs, dry 8-10	4	*	68	19	7	290	0.162	0.116	0.0029	50-90	80-180	45	0	1.72 mg. niacin	12
13. Grapefruit ½ (4 in. diam.)	*	*	10	89	*	44	0.021	0.020	0.0003	21	50-100	12-20	35-45		13

°Carbohydrate value includes fiber.

8° "Water-packed" foods have a carbohydrate content approximately 4% less than the fresh or "juice-packed." Added sugar may increase carbohydrate content markedly, depending upon sugar content of the syrup. Juice packed may be calculated as the fresh fruit itself.



TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL

EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 OZ.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (i. u.)	THIAMINE ( $\mu$ g.)	RIBOFLAVIN ( $\mu$ g.)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
Fruits—Continued															
14. Grapes, American 20-25	1	1	14	82	•	78	0.015	0.021	0.0007	2-60	37-80	30-60	2-3		14
15. Honeydew melon 1 $\frac{1}{4}$ (5 in. diam.)	•	•	4	63	•	18									15
16. Huckleberries $\frac{2}{3}$ c.	•	•	14	84	1	68	0.025	0.020	0.0069	20-80	45	15	4-10		16
17. Lemon 1 (2 $\frac{1}{2}$ in. long)	1	1	8	89	1	44	0.022	0.011	0.0006	0	30-90	4	45-60		17
18. Loganberries 1 $\frac{1}{8}$ c.	1	1	14	83	2	69	0.035	0.022	0.0014	+	+	35	35-40		18
19. Muskmelon $\frac{1}{4}$ (5 in. diam.)	1	•	5	93	1	27	0.016	0.015	0.0004	200- 2,400	50-65	75	26-34		19
20. Orange 1 (2 $\frac{1}{2}$ in. diam.)	1	•	10	87	1	45	0.024	0.018	0.0004	50-400	75-145	28-90	50-66	16 vitamin B <sub>6</sub> curative units 0.17-0.22 mg. niacin 70 $\mu$ g. pantoic acid	20
21. Papaya $\frac{1}{4}$ (5 in. diam.)	1	•	9	89	1	43	0.018	0.013	0.0003	2,000- 3,000	15-30	83	35-55		21
22. Peach 1 medium	1	•	12	87	1	51	0.010	0.019	0.0003	100- 2,000	20-70	45-60	7-16	0.9-1 mg. niacin White 0-100A Yellow 1000-2000A	22
23. Pear 1 medium	1	•	14	83	1	70	0.015	0.018	0.0003	10-15	30-95	20-150	3-5	0.14 mg. niacin	23
24. Pineapple, fresh 1 slice (3 in. thick) (2 $\frac{3}{4}$ c. cubed)	•	•	13	85	•	58	0.016	0.011	0.0004	40-60	80-125	50-80	8-18		24
25. Plums 3	1	•	12	86	1	56	0.020	0.027	0.0006	100-115	48-200		4-7	0.56 mg. niacin	25
26. Prunes, dried 5-6	2	0	73	22	2	310	0.058	0.085	0.0029	800- 2,400	60-225	20-200	0-8		26
27. Raisins $\frac{3}{4}$ c.	3	3	76°	15	0	355	0.060	0.102	0.0030	10-100	100-200	125	0	0.6 mg. niacin	27
28. Raspberries, red $\frac{3}{4}$ c.	1	1	12	83	3	67	0.041	0.098	0.0009	150	25-30		20-30		28
29. Rhubarb 1 c. dried	•	•	3	95	1	18	0.044	0.031	0.0010	100	25		12-24		29

	1	1	7	90	1	41	0.041	0.028	0.0008	60-90	25	50-80	0.22-0.26 mg. niacin	30
10-15 (2 $\frac{3}{8}$ c.)														
31. Tangerines 2 small (2 in. diam.)	1	•	9°	89		43	0.041	0.018	0.0003	350	120	25-50		31
32. Watermelon 1 slice (21 $\frac{1}{2}$ ×21 $\frac{1}{2}$ ×1 in.)	•	•	6	92	1	31	0.007	0.013	0.0002	50-125	30-40	8-12		32

Fruit Juices<sup>11</sup>

1. Apple 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	•	0	13	87	0	50								1
2. Grapefruit 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	•	•	10	89	0	42							0.14-0.15 mg. niacin	2
3. Grape 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	•	0	19	81	0	76	0.011	0.010	0.0003		30-60			3
4. Lemon 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	1	•	9	90	0	42	0.022	0.011	0.0006		30-90	50-60		4
5. Lime 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	1	0	8	91	0	33								5
6. Orange 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	1	•	13	86	0	55	0.018	0.014	0.0003	280	50-100	10-18	0.08 mg. B <sub>6</sub> 1.5 mg. biotin 0.004 mg. inositol 0.21 mg. niacin 0.07 mg. pantothenic acid	6
7. Pineapple 3 $\frac{1}{2}$ oz. (approx. $\frac{2}{5}$ c.)	•	•	13	88	0	53	0.018	0.01	0.0001	40-60	50-100	20-30	0.14 mg. niacin	7

Meats<sup>12</sup>

1. Beef, brain $\frac{1}{8}$ of a brain	11	9	1	78		127				180	150-250			1
2. Beef, chuck, canned 1 slice (1 $\frac{1}{2}$ ×3×3 $\frac{1}{2}$ in.)	19	16	0	65		218	0.013	0.204	0.003					2
3. Beef, corned, canned 2 slices (2×4× $\frac{1}{2}$ in.)	23	19	0	71		274	0.013	0.119	0.0098		+	++		3

<sup>11</sup>Carbohydrate value includes fiber.

<sup>12</sup>Use mineral and vitamin values of fruits for fruit juices. Addition of sugar increases carbohydrate value of fruit juices 1 to 3%.

<sup>13</sup>Meat of firm texture, no bone, and little fat averages 6 to 9 cubic inches per 100 Gm. Meat of loose texture averages 9 to 12 cubic inches per 100 Gm.

The average values for the mineral content of meats, as given by Sherman, are: 0.058 Gm. calcium, 1.078 Gm. phosphorus, and 0.015 Gm. iron per 100 Gm. protein. In as much as "meats" are, on the average approximately 20% protein, the values 0.12 Gm. calcium, 0.218 Gm. phosphorus, and 0.003 Gm. iron may be used for 100 Gm. meat.

On the average lean muscle of meat contains 300  $\mu$ g. riboflavin, 10 mg. niacin, 25 curative units vitamin B<sub>6</sub>, and 1 mg. pantothenic acid per 100 Gm. meat.

Elvehjem and his associates report the average vitamin retention in cooked pork, ham, and loins as: in the meat alone, 70% of the thiamine in roasting and broiling and 50% in braising; for niacin in roasting and broiling 85%, in braising 65%. For riboflavin there is 85% retention with any of the methods. The total retention in the meat plus the drippings is about the same for all methods—an average of 70% for thiamine and at least 90% for riboflavin and niacin. Appreciable amounts of each of the vitamins are found in the drippings, particularly from braised loin cuts.

A wide variation was found in the thiamine and riboflavin content of different pork carcasses. The niacin content is more constant.

TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL  
EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 Oz.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (I. U.)	THIAMINE ( $\mu$ g)	RIBOFLAVIN ( $\mu$ g)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
MEATS—Continued															
Beef—Continued															
4. Beef, dried 1 c. loosely packed	30	7	0			178	0.018	0.326	0.0045		+	++			4
5. Beef, heart 1 slice (2×3×1 in.)	17	4	1	78		104				+	400-600	750-900		7-8 mg. biotin	5
6. Beef, kidney ½ c. diced	15	8	1	75		136	0.010	0.180	0.004	500- 1,000	150-315	1,700- 2,400		92 mg. biotin 333 mg. choline 7-8 mg. niacin	6
7. Beef, liver 1 slice (3¼×3×½ in.)	20	3	6	70		132	0.011	0.368	0.0082	5,000- 10,000	300-420	1,800- 3,700		45 I. U. vitamin D 88-118 mg. biotin 630 mg. choline 25-30 mg. niacin	7
8. Beef, loin, medium lean 1 slice (4×4×½ in.)	17	25	0	57		293	0.013	0.204	0.003	10-60	110-210	180-400		6-8 mg. niacin	8
9. Beef, porterhouse, medium lean 2 slices (3×2×1 in.)	22	20	0	58		273	0.013	0.204	0.003					0.4 mg. B <sub>6</sub> 3.4 mg. biotin 82 mg. choline in "beef" 1 mg. pantothenic acid	9
10. Beef, rib, 82% lean 1 slice (5×2½×¼ in.)	17	23	0	59		277	0.013	0.204	0.003						10
11. Beef, round, 87% lean 2 slices (3½×2×1 in.)	19	13	0	67		194	0.013	0.204	0.003					3-6 mg. biotin 95 mg. choline 7-9 mg. niacin	11
12. Beef, rump, 67% lean 2 slices (½×2×1 in.)	15	31	0	53		341	0.013	0.204	0.003						12
13. Beef, tongue, medium lean 5 slices (¼ in. thick)	19	9	•	68		158	0.030	0.119	0.0069		300	200		3.3 mg. biotin 7 mg. niacin	13
14. Beef, tripe 3½ oz.	19	2	0	79		94									14
Lamb															
1. Lamb, chops, loin 3 medium	18	28	0	51		321	0.01	0.22	0.003		180-330	280		6 mg. biotin 107 mg. choline	1
2. Lamb, leg, medium fat 2 slices (1×4½×½ in.)	19	17	0	64		235	0.011	0.207	0.0015		200-300	170-280		4-8 mg. niacin	2
3. Mutton, chops, loin, medium fat 2 medium	16	33	0	50		375	0.014	0.216	0.002		200-300	280			3





TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL  
EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 Oz.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (I. U.)	THIAMINE (μg)	RIBOFLAVIN (μg)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
Meats—Continued															
Sausage															
1. Beef and pork link, all meat 6 sausages (3×3¼ in.)	20	41	0	45		416									1
2. Blood 6 slices (¼ in. thick)	15	35	0	47		371									2
3. Bologna, all meat 6 slices (½ in. thick)	14	18	0	64		218	0 003	0 060	0 0028	+	525	+			3
4. Frankfurters, all meat, 8 per pound 1½ sausage	14	21	0	61		244	0 01	0 22	0 0025		+	+			4
5. Frankfurters, cereal added, 8 per pound 1½ sausage	15	14	0	64		201									5
6. Liver 4 slices (¼ in. thick)	17	21	2	59		258									6
7. Pork, no cereal 6 sausages (3×¾ in.)	11	45	0	42		446	0 002	0 027	0 001		350				7
8. Salami 6 slices (¼ in. thick)	24	37	0	31		427									8
Veal															
1. Veal cutlet 2 slices (2×3×½ in.)	20	9	0	70		159	0 014	0 229	0 003		150	345-375		6-18 mg. niacin	1
2. Veal, loin 2 slices (2×3×½ in.)	19	11	0	69		176								113 mg. choline	2
3. Veal, sweetbreads 3 average	20	3	0	75		106									3
The small amount of ascorbic acid originally present in muscle tissue is lost before the meat is consumed; therefore, meat may be considered as devoid of vitamin C															
Miscellaneous															
1. Beverages, carbonated 100 c.c. (⅔ c.)	0	0	9	91	0	36									1
2. Candies, caramel 6-8	2	12	78	7	0	428									2
3. Candies, creams, chocolate 6-8	4	14	72	9	0	430									3
4. Candies, fudge 100 g. (2¼ × 5 × ½ in.)	2	4	88	5	0	336	0 04	0 06	0 0004						4

[illegible]



TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL  
EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 Oz.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (I. U.)	THIAMINE (μg.)	RIBOFLAVIN (μg.)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
N U T S															
1. Almonds 90-100 (¾ c.)	19	54	20	5	3	640	0.239	0.465	0.0039	580	120-240	600	Nuts are entirely devoid of ascorbic acid	1.8 mg. niacin	1
2. Brazil 15-20	14	66	11	5	2	695	0.123	0.602	0.0028	10	1,000				2
3. Cashew 90-100	20	47	26	4	1	609	0.048	0.480			+	190			3
4. Chestnut, fresh 40-50	6	5	42	53	1	242	0.034	0.093	0.0007		170-270			1.2 mg. niacin	4
5. Coconut, dry, shredded 1½ c.	4	41	44	3	4	566	0.059	0.155	0.005		100-200	++			5
6. Hazelnut (filbert) 90-100	13	61	18	6	3	670	0.287	0.354	0.0041	100-440	300-660				6
7. Peanuts, roasted 125-140 (¾ c. shelled)	27	44	24	3	2	600	0.071	0.399	0.0020	360	500- 1,000	200-500		Peanuts and peanut butter contain 1,600 curative units vitamin B <sub>6</sub> ; peanut meal contains 13 mg. niacin and 4-6 mg. pantothenic acid	7
8. Peanut butter 5 tbs.	26	48	21	2	2	619	0.071	0.399	0.0020	360	500- 1,000	200-500			8
9. Pecans 90-100 (¾ c. shelled)	9	73	13	3	2	747	0.089	0.335	0.0026	100-400	150- 1,000				9
10. Walnuts, Persian or English 90-100	15	64	16	3	2	702	0.089	0.358	0.0021	100-150	300-600			0.8 mg. pantothenic acid	10

SOUP, AVERAGE COMMERCIAL, UNDILUTED

1. Asparagus 2/3 c. (approx. ½ can)	1	1	7	88	•	45								Usual dilution—equal parts of concentrated soup and water or other liquid.	1
2. Bean 2/3 c. (approx. ½ can)	6	2	14	75	1	100									2
3. Beef 2/3 c. (approx. ½ can)	6	1	8	82	•	68									3
4. Bouillon 2/3 c. (approx. ½ can)	3	0	1	94	0	16									4
5. Celery 2/3 c. (approx. ½ can)	1	2	7	87	•	48									5

6. Chicken 2/3 c. (approx. 1/3 can)	1	1	5	89	*	33							6
7. Clam Chowder 2/3 c. (approx. 1/3 can)	3	4	19	80	*	90							7
8. Pea 2/3 c. (approx. 1/3 can)	5	2	12	78	1	85	0.092	0.088	0.0008	300-400	10-15	100-200	8
9. Tomato 2/3 c. (approx. 1/3 can)	2	2	9	84	*	58							9
10. Vegetable 2/3 c. (approx. 1/3 can)	3	2	10	80	*	68							10

SPECIAL FOODS

(Exact analyses are taken from manufacturers' literature and from *Accepted Foods*.)

[illegible]

TABLE LII—CONT'D

## COMPOSITION OF FOODS, EDIBLE MATERIAL

EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 OZ.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIE (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (i. u.)	THIAMIN <sup>1</sup> (μg.)	RIBOFLAVIN (μg.)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
<b>SPECIAL FOODS—Continued</b>															
<i>Strained Foods (Heinz)—Cont'd</i>															
9. Peas and pineapple ½ c.	0.4	0.4	12.9		1.0	56	0.013	0.010	0.0011	165	36	42	3		9
10. Peas ½ c.	4.9	0.5	8.8		1.0	60	0.012	0.83	0.0016	1,680	126	122	14		10
11. Prunes ½ c.	1.1	0.2	28.2		0.8	120	0.033	0.032	0.0018	200	42	142	9		11
12. Spinach ½ c.	2.2	0.5	1.5		0.7	17	0.057	0.039	0.0010	5,530	29	105	18		12
<b>SUGARS</b>															
1. Brown 10 tbs. or ½ c.	0	0	96.0	3.0	0	384									1
2. Corn syrup 6 tbs. or ¾ c.	0	0	86.0	14.0	0	344	0.0595	0.007	0.0127						2
3. Granulated ¾ c.	0	0	100.0	0	0	400									3
4. Honey, strained 5 tbs.	0	0	80	20	0	320	0.004	0.019	0.0007		3-9	35-80	1-4	2-0.4 mg. B <sub>6</sub> 0.2 mg. pantothenic acid	4
5. Maple 1 piece (1¾×1¼×½ in.)	0	0	90	8	0	360									5
6. Maple syrup 6 tbs. or ¾ c.	0	0	74	26	0	296	0.107	0.013	0.003						6
7. Molasses, cane ½ c.	2	0	69	24	0	287	0.258	0.030	0.0093					Cane molasses is a good source of vitamin B <sub>6</sub> and contains pantothenic acid and vita- min B <sub>12</sub> ; beet molasses is devoid of all vitamins	7
<b>VEGETABLES</b>															
1. Artichokes, French or Globe 4 small heads	3	0	12	84	3	60	0.040	0.094	0.001	150-300	75-180	30	9		1
2. Asparagus, canned 8 tips or ½ c.	2	0	3	94	0	20	0.021	0.040	0.0010	1,000	190		60	128 mg. choline 1.2 mg. niacin	2
3. Asparagus, fresh, green 5 stalks, 8 tips or ½ c.	2	0	4	93	1	24	0.021	0.040	0.0010	300-900	150-180	100-150	15-40	Pantothenic acid +	3



4. Beans, baked, canned with pork $\frac{1}{4}$ c. scant	6	2	19	71	1	117	0.062	0.185	0.0020	40-70	132	+	0	4
5. Beans, butter, fresh $\frac{2}{3}$ c.	2	0	8	89	1	40	0.055	0.50	0.0012	350	87	100	340 mg. choline 0.3 mg. niacin	5
6. Beans, dried, navy $\frac{1}{2}$ c.	22	2	62	11	4	350	0.148	0.463	0.0105	0-74	300-500	1,200	400 curative units of vitamin B <sub>6</sub>	6
7. Beans, kidney, canned $\frac{1}{2}$ c.	6	0	16	76	1	92	0.044	0.139	0.003				0	7
8. Beans, Lima, dried $\frac{2}{3}$ c.	21	1	62	13	4	341	0.072	0.386	0.0097		450-600	790	0	8
9. Beans, Lima, fresh $\frac{2}{3}$ c.	8	0	24	67	2	131	0.028	0.133	0.0024	500	250-350	250-900	0.8-1 mg. niacin	9
10. Beans, soy, dried $\frac{1}{2}$ c.	35	13	7	8	5	350	0.071	0.201	0.0020	100	1,455	900	0	10
11. Beans, soy, fresh $\frac{3}{4}$ c.	13	7	4	67	2	132				130-700	525- 1,200	300	340 mg. choline 4.8 mg. niacin	11
12. Beans, string, canned $\frac{1}{2}$ c.	1	0	3	94	1	16	0.055	0.050	0.0012	++	++		Pantothenic acid +	12
13. Beans, string, fresh $\frac{2}{3}$ c. (1 in. pieces)	2	0	8	89	1	40	0.055	0.050	0.0012	600- 1,800	55-95	65-150	10-20	13
14. Bean sprouts (mung beans) 1 c. scant	3	0	4	92	1	28	0.042	0.054	0.0010				+++	14
15. Beets, fresh $\frac{1}{2}$ c. or 2 beets (2 in. diam.)	2	0	10	88	1	48	0.029	0.042	0.0008	15	51	23	Pantothenic acid + 13 cura- tive units vitamin B <sub>6</sub>	15
16. Beet greens 3 c. ( $\frac{1}{2}$ c. cooked)	2	0	6	90	1	32	0.094	0.040	0.0032	++		82-625	35	16
17. Broccoli, flower stalks $\frac{2}{3}$ c.	3	0	6	90	1	36	0.064	0.105		3,000- 9,000	80-100	200-500	1.4 mg. pantothenic acid	17
18. Brussels sprouts 8-10 or $\frac{2}{3}$ c.	4	•	8	85	1	53	0.027	0.121	0.0012	200-500	171	90-180	13-50	18
19. Cabbage, fresh $\frac{2}{3}$ c. shredded ( $\frac{1}{2}$ c. cooked)	1	•	4	92	1	20	0.046	0.034	0.0004	30-80	27	44	0.3 mg. B <sub>6</sub> 251 mg. choline 0.1-0.3 mg. niacin 0.23 mg. pantothenic acid	19
20. Carrots $\frac{1}{2}$ - $\frac{3}{4}$ c. diced	1	•	8	88	1	40	0.045	0.041	0.0006	2,200- 4,000	60-140	75-125	95 mg. choline 0.2-0.35 mg. niacin 0.2 mg. pantothenic acid	20
21. Cauliflower $\frac{1}{2}$ - $\frac{3}{4}$ c.	2	•	4	92	1	31	0.025	0.060	0.0009	30-60	130-200	150-220	0.5-0.6 mg. niacin	21

TABLE LII—CONT'D

## COMPOSITION OF FOODS, EDIBLE MATERIAL

EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 Oz.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (I. U.)	THIAMINE ( $\mu$ E)	RIBOFLAVIN ( $\mu$ E)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
VEGETABLES—Continued															
22. Celery, bleached 1 heart with 3 stalks	1	*	3	94	1	22	0.078	0.046	0.0006	5-50	36	35	6-8	0.18-0.26 mg. niacin	22
23. Chard, leaves 3 c. shredded ( $\frac{1}{2}$ c. cooked)	3	*	4	91	1	33	0.100	0.050	0.0031	1,300- 2,700		140	10-43		23
24. Corn, canned $\frac{1}{2}$ c. or 2 ears (4 in. long)	3	1	19	76	*	96	0.006	0.098	0.0005		100		6		24
25. Corn, yellow sweet, fresh 2 ears (4 in. long), $\frac{1}{2}$ c.	3	1	20	74	1	108	0.006	0.103	0.0005	500	120-150	121	8-11	0.8 mg. pantothenic acid	25
26. Cucumber $\frac{1}{2}$ c. sliced	1	*	2	96	1	14	0.006	0.018	0.0003	15-50	45-90	24-150	2-13	0.1-0.3 mg. niacin	26
27. Dandelion greens 3 c. shredded ( $\frac{1}{2}$ c. cooked)	3	1	7	86	2	52	0.113	0.043	0.0035	12,500- 27,000	150-225	225	5-40		27
28. Eggplant 4 avg. slices ( $\frac{3}{4}$ c. diced)	1	1	5	93	1	28	0.011	0.031	0.0005	20-100	40-100	30	1-9	0.6 mg. niacin	28
29. Endive, French 15-20 stalks	2	*	4	93	*	24	0.104	0.039	0.0012	1,000	75	50-70	8-12		29
30. Escarole (endive) $\frac{1}{2}$ head (2-4 stalks)	2	*	3	93	1	24	0.104	0.039	0.0012	13,000- 18,000	99-120	75-200	6-20		30
31. Kale (collards) 1 c. cooked	4	*	6	87	1	50	0.181	0.067	0.0025	13,000- 20,000	120-190	400-600	50-100	0.3 mg. pantothenic acid	31
32. Kohlrabi $\frac{1}{2}$ - $\frac{3}{4}$ c. diced	2	*	6	90	1	36	0.078	0.057	0.0006	10,000	40-70		40-80		32
33. Leeks 3-4 (5 in. long)	2	*	7	98	1	45	0.058	0.056	0.0006		90-150		10-20		33
34. Lentils, dried, whole $\frac{1}{2}$ c.	25	1	57	11	3	345	0.102	0.383	0.0086		300-600	200-315			34
35. Lettuce $\frac{1}{4}$ - $\frac{1}{2}$ head or 12 medium leaves	1	*	2	95	1	18	0.017	0.028	0.0015	70-100 head 700- 5,000 green leaves	75	45	4-21	Romaine, 1,000 I. U. vitamin A	35
36. Mushrooms $\frac{1}{2}$ c. canned (1 c. fresh)	?	*	?	91	1	?	0.014	0.098	0.0007	0	100-200		3-6		36

37. Okra 5 pods	2	*	6	90	1	39	0.072	0.053	0.0006	300-600	126		10	37
38. Onions 5-6 small or 2 large ( $\frac{1}{2}$ c. sliced)	1	*	10	88	1	49	0.041	0.047	0.0005	0-5,000	30	28-62	10	0 1-0.2 mg. niacin 0.1 mg. pantothenic acid Green onions, 5,000 I. U. vitamin A; mature ones, 0
39. Parsley 1 bunch (5 in. diam.)	4	1	8	84	2	60			0.0032	30,000			75-150	39
40. Parsnips $\frac{1}{2}$ c. cubed	2	1	16	79	2	83	0.060	0.076	0.0008	+	120-190	60-90	8-20	40
41. Peas, canned, drained $\frac{1}{2}$ c.	3	*	9	85	1	55	0.011	0.106	0.0024	++	200-300	80-200	2-10	41
42. Peas, dried, split $\frac{1}{2}$ c.	25	1	60	10	1	354	0.077	0.411	0.0057	50	300-620	250-380	+	42
43. Peas, green, fresh $\frac{3}{4}$ c.	5	*	10	81	2	73	0.023	0.127	0.0021	1,000-1,300	270-495	130-250	15-25	43
44. Peppers, green 1 (3-4 in. long)	1	*	4	92	1	29	0.012	0.028	0.0004	5,000	20-30	140	90-180	44
45. Peppers, red 1 (3-4 in. long)	1	1	7	89	2	44				5,000	30	138	200-230	45
46. Potato chips 4 c.	7	37	49	3	*	557	0.005	0.021	0.0004					46
47. Potatoes, fresh 1 small ( $2\frac{1}{2}$ in diam.) ( $\frac{3}{4}$ c. rice)	2	*	19	78	*	85	0.013	0.053	0.0010	30-50	95-165	40-80	7-15	47
48. Potatoes, sweet (yam) $\frac{1}{2}$ medium or 1 small	2	*	27	69		120	0.030	0.052	0.0010	1,000-5,000	90-135	80-100	7-20	48
49. Pumpkin, canned $\frac{1}{2}$ c.	1	*	7	90	1	38								49
50. Pumpkin, fresh $\frac{3}{4}$ c. diced	1	*	6	91	1	36	0.023	0.046	0.0009	2,500	45	45-100	5	50
51. Radishes 10 medium or 15 small	1	0	2	46	*	11	0.031	0.031	0.0008		50-100	30	12-25	51
52. Rutabagas, yellow (Swede) $\frac{2}{3}$ c. ( $\frac{1}{2}$ in. cubes)	1	*	8	89	1	41	0.074	0.056	0.005	25	75		150	52
53. Salsify (vegetable oyster) $\frac{1}{2}$ c. diced	4	1	14	79	2	85								53
54. Sauerkraut $\frac{3}{4}$ c.	1	*	4	91	1	27				2	30		0-10	54



TABLE LII—CONT'D  
COMPOSITION OF FOODS, EDIBLE MATERIAL  
EXPRESSED AS PERCENTAGES OR GRAMS PER 100 GRAMS (APPROX. 3.5 Oz.) EXCEPT FOR VITAMINS, WHICH ARE EXPRESSED ON VARIOUS BASES

FOODS	PROTEIN	FAT	CARBO- HYDRATE	WATER	FIBER	CALORIES (APPROX.)	CALCIUM	PHOS- PHORUS	IRON	VITAMIN A (I. U.)	THIAMINE ( $\mu$ g)	RIBOFLAVIN ( $\mu$ g)	ASCORBIC ACID (mg.)	REMARKS	FOOD NOS. SEE FIRST COL.
VEGETABLES—Continued															
55. Spinach, canned $\frac{1}{2}$ c.	2	•	3	92	1	28									55
56. Spinach, fresh 3 c. ( $\frac{1}{2}$ c. cooked)	2	•	2	93	1	25	0.078	0.046	0.0026	25,000	105	160	40-75	238 mg. choline 0.7 mg. niacin 0.1-0.2 mg. pantothenic acid 66 curative units vitamin B <sub>6</sub>	56
57. Squash, summer $\frac{3}{4}$ c. diced ( $\frac{1}{2}$ c. cooked)	1	•	3	95	1	19	0.018	0.015	0.0004	200-400	42	52-80			57
58. Squash, winter (Hubbard) $\frac{3}{4}$ c. diced ( $\frac{1}{2}$ c. cooked)	2	•	7	89	1	44	0.019	0.028	0.0006	2,000- 4,000	48	46-81	3	1 mg. niacin	58
59. Tomato, canned $\frac{1}{2}$ c.	1	•	3	92	•	21	0.007	0.021	0.0006	500- 1,200	70-115	37-63	21-24		59
60. Tomato, fresh 1 medium (2 $\frac{1}{2}$ in. diam.)	1	•	3	94	1	23	0.007	0.021	0.0006	500- 1,200	50-115	37-63	18-24	24 curative units vitamin B <sub>6</sub> 0.2-0.6 mg. niacin 0.1 mg. pantothenic acid	60
61. Tomato, green 1 medium	1	•	3	95	•	20	0.011	0.029	0.0004						61
62. Tomato juice, canned $\frac{2}{3}$ c.	1	•	4	94	•	23	0.007	0.015	0.0004	500- 1,200	70-115	37-63	12-25	Salt 0.5%	62
63. Turnip, white $\frac{2}{3}$ c. ( $\frac{1}{2}$ in. cubes)	1	•	6	91	1	35	0.056	0.047	0.0005	10-20	65-95	50-100	20-30	0.25 mg. pantothenic acid	63
64. Turnip greens 3 c. ( $\frac{1}{2}$ c. cooked)	3	•	4	90	1	37	0.279	0.049	0.0035	13,000- 18,000	138-160	750	20-60		64
65. Water cress 2 $\frac{1}{2}$ c.	2	•	3	94	1	23	0.157	0.046	0.0030	800- 4,000	100-150	150-300	43-66		65

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TABLE LIII\*

SODIUM AND POTASSIUM CONTENT OF FOODS. ANALYSES MADE ON EDIBLE PORTIONS OF UNPROCESSED FOODS EXCEPT AS OTHERWISE DESIGNATED

FOOD	SODIUM mg./100 gm.	POTASSIUM mg./100 gm.	FOOD	SODIUM mg./100 gm.	POTASSIUM mg./100 gm.
All-Bran cereal	1400	1200	Candy, continued		
Almond			Gum drop	41	18
Raw	3	690	Marshmallow	41	6
Roasted in oil, salted	160	710	Milk chocolate	86	420
Anchovy paste	9800	200	Necco Wafers	5	2
Apples			Peppermint patty, Schrafft's	10	110
Juice (sweet cider), bottled	4	100	Sweet chocolate	35	230
Mackintosh, less skin and core	0.2	90	Cantaloupe	12	230
Sauce, canned	0.3	55	Carrots		
Apricot			Canned	280	110
Canned in sirup	2	65	Scraped and trimmed	31	410
Dried	11	1700	Cashew nuts		
Raw, with skin	0.6	440	Roasted in oil, salted	200	560
Artichoke, globe	43	430	Catchup, tomato	1300	800
Asparagus			Catfish (fiddler), Ohio River	60	330
Spears, canned	410	130	Cauliflower		
Tips, fresh	2	240	Buds	24	400
Avocado	3	340	Buds, frozen	22	290
Bacon			Caviar, salmon, canned	2200	180
Fried crisp	2400	390	Celery Stalks, less leaves	110	300
Baking powder			Cereals, dry		
Alum type	10,000	150	Bran		
Phosphate type	9000	170	All-Bran	1400	1200
Tartrate type	7300	5000	Crude, unsalted	15	980
Banana	0.5	420	Corn flakes	660	160
Barley, pearled	3	160	Farina		
Beans			Cream of Wheat, plain	2	86
Baked, Navy			Cream of Wheat, quick-cooking,		
With pork and tomato sauce, canned	480	210	enriched	90	84
With tomato sauce, canned	400	140	Grape-Nuts	660	230
Dry, Navy	1	1300	Pabulum	620	380
Green, in pods			Rolled Oats	2	340
Canned	410	120	Ry-Krisp	1500	600
Fresh	0.9	300	Wheat		
Lima			Flakes	1300	320
Canned	310	210	Germ, malt-flavored, Zing	9	780
Fresh	1	680	Instant Ralston	1	360
Frozen	310	580	Maltes	4	250
Beef			Muffets	4	300
Corned	1300	60	Pettijohn's	2	380
Dried	4300	200	Puffed	4	340
Lean, koshered, raw	1600	290	Shredded	2	330
Lean, raw	51	360	Wheatena	2	380
Beer	8	46	Chard		
Beets			Large leaves	210	720
Canned	36	120	Small leaves	84	380
Greens, fresh	130	570	Cheese		
Raw	110	350	American Swiss	710	100
Blackberry	0.2	150	Cheddar	700	92
Blueberry	0.6	89	Cottage	290	72
Bouillon cube	21,000	100	Cream, Philadelphia	250	74
Brain, pig	150	340	Process	1500	80
Bran, wheat, crude	15	980	Whey, Velveeta	1600	270
Brazil nuts			Cherries		
Raw	1	670	Sweet		
Bread			Dark, raw	1	260
Boston brown, with raisins	280	360	Canned in sirup	0.8	77
Low-sodium—4 laboratory samples	3	94	Chestnut	2	410
Low-sodium cinnamon roll	2	120	Chicken, raw		
Low-sodium—14 commercial "salt-free" breads:			Breast meat	78	320
Maximum	76	200	Leg meat	110	250
Minimum	4	72	Chocolate		
Average	28	120	Sirup, Hershey	60	130
Rye and wheat	590	160	Unsweetened	4	830
White, enriched	640	180	Cider, sweet (apple juice), bottled	4	100
Whole wheat	930	230	Citron, candied	290	120
Whole wheat and white	620	250	Clam	180	240
Broccoli			Cocoa		
Fresh	16	400	Dutch process	57	3200
Frozen	13	250	Plain, Hershey	5	1400
Brussels sprouts			Coconut		
Fresh	11	450	Dry, shredded	16	770
Frozen	9	300	Meat	29	320
Butter			Milk	53	190
4 Indiana samples	880	23	Cod		
Unsalted	5	4	Raw	60	360
Buttermilk, cultured	130	140	Frozen fillets	400	400
Cabbage	5	230	-liver oil	0.1	0
Candy			Salted, dried	8100	160
Bar, Baby Ruth	170	300	Coffee		
Bar, Milky Way	220	150	Instant, Nescafe, dry	84	3100
Bar, Oh Henry	76	420	Roasted		
			Decaffeinated, Sanka, dry	6	2000
			Regular, dry	2	1600

\*Selected from Table 1 of Bills, C. E., McDonald, F. G., Neidermier W., and Schwartz, M. C.: Sodium and Potassium in Foods and Waters, J. Am. Dietet. A. 25:304 1949.



TABLE LIII—CONT'D

FOOD	SODIUM mg./100 gm.	POTASSIUM mg./100 gm.	FOOD	SODIUM mg./100 gm.	POTASSIUM mg./100 gm.
Cookie, salt-free, Betty Bakerite	12	240	Horse-radish, prepared	96	290
Corn Meal, yellow, enriched, degerminated	0.7	120	Ice Cream	100	90
Oil	0.2	0.1	Jam, grape	7	78
Popcorn, popped and oiled	3	240	Kale, leaves and midribs	110	410
Popcorn, popped, oiled, and salted	2000	240	Kidney, beef	210	310
Starch	4	4	Kumquat, pulp and rind, less seeds	7	230
Sweet			Lamb		
Yellow			Chop, lean, raw	98	340
Canned	210	200	Leg, lean, raw	78	380
Frozen	9	190	Lard	0.3	0.2
Milk stage	0.4	370	Lemons, pulp and juice	0.7	130
Peas, fresh, shelled	2	560	Lentils, dry	3	1200
Peas, canned	1000	110	Lettuce		
Peas, cracked			Head	12	140
Graham	710	330	Leaf	7	230
Rye, Ry-Krisp	1500	600	Lime, pulp and juice	1	100
Soda	1100	120	Liver, raw		
Strawberry			Calf	110	380
Raw	1	65	Goose	140	230
Sauce, canned	1	17	Pig	77	350
cream, whipping, 32% fat	40	56	Turkey	51	160
Cucumber, less parings	0.9	230	Lobster, boiled in tap water	210	180
Currants			Lonolac, dry	13	1300
Red	2	160	Macaroni, plain, dry	1	160
Curry powder	45	1300	Maple sirup	14	130
Endive greens	76	430	Marmalade, orange	13	19
Egg, white, semi-dry, California	1	790	Matzo		
Extrose	1	0.4	Meal	4	130
Fish, domesticated, raw			Passover (Passover bread)	1	140
Breast meat	68	360	Mayonnaise	590	25
Leg meat	96	210	Meat extract, flavored	11,000	6000
Egg			Milk		
Whites only	110	100	Cow's		
Whole	81	100	Buttermilk, cultured	130	140
Yolks only	26	100	Condensed, sweetened	140	340
Eggplant, less skin	0.9	190	Evaporated	100	270
Endive greens	18	400	Fat	0.4	0.3
Beans			Skim	52	150
Canned in sirup	1	105	Whole		
Dried	34	780	Dry	410	1100
Raw	2	190	Liquid	50	140
Almonds (hazelnut)	1	560	Malted, dry	440	720
Flour			Molasses, cane	80	1500
Bleached			Mushrooms		
Enriched, Gold Medal	1	86	Canned	400	150
Enriched, phosphated	13	78	Raw	5	520
Buckwheat	1	680	Mustard		
Gluten	2	24	Greens	48	450
Rye, dark	1	860	Powder	3	840
Self-rising	1500	90	Prepared paste	1300	130
Untreated, high-extraction	1	120	Nectarine, less skin	2	320
Whole wheat (Graham)	2	290	Oats, rolled (oatmeal), dry	2	340
Fruit cocktail, canned in sirup	9	160	Okra, fresh	1	220
Gelatin			Oleomargarine	1100	58
Dessert, Flavored, Jell-O	330	210	Olives		
Plain	36	22	Green, pickled	2400	55
Spaghetti	29	1100	Oil	0.2	0.2
Spiced, turkey	58	170	Ripe, pickled	980	23
Wheat flour	2	24	Onion, less tops and dry skins	1	130
Peas, raw			Oranges		
Breast meat	76	420	Juice, unsweetened, canned	0.5	190
Leg meat	96	420	Pulp and juice	0.3	170
Cherry			Temple, pulp and juice	3	220
Raw	0.7	87	Oyster, raw	73	110
Apples			Pancreas, pig, raw	57	240
Concord, less seeds and skin	3	84	Parsley, fresh	28	880
Emperor, less seeds, with skin	4	180	Parsnip, scraped and trimmed, fresh	7	740
Jam	7	78	Peaches		
Juice, Concord, sweetened, bottled	1	120	Canned in sirup	5	31
Thompson Seedless, with skin	4	180	Dried	12	1100
Tokay, less seeds, with skin	0.7	160	Raw, less skin	0.5	160
Grapefruit			Peanuts		
Fresh	0.5	200	Butter	120	820
Juice, sweetened, canned	0.4	150	Oil	0.2	0.1
Savory flavoring, Kitchen Bouquet	86	280	Roasted		
Alfalfa			Dry, with skin	2	740
Alfalfa	56	540	In oil and salted,		
Beef, frozen	460	500	with skin	460	700
Beef, raw	1100	340	Pears		
Beef, corned beef, canned	540	200	Bartlett	8	52
Beef	90	160	Canned in sirup	2	100
Turkey	69	240	Raw, less skin and core		
Vegetable, canned	250	22	Peas		
Vegetable	7	10	Canned, less liquor	270	96
			Dry, split	42	880
			Fresh	1	370

TABLE LIII—CONT'D

FOOD	SODIUM mg./100 gm.	POTASSIUM mg./100 gm.	FOOD	SODIUM mg./100 gm.	POTASSIUM mg./100 gm.
Pecan, raw	0.3	420	Sirup		
Pepper (spice)			Maple	14	130
Black	16	880	Sorghum	21	600
Red	46	2400	Table, corn-and-cane, Karo Crystal		
White	5	48	White	68	4
Peppermint extract	0.3	5	Soft drinks		
Peppers, green, empty pods	0.6	170	Carbonated water		
Pickle, dill	1400	200	Canada Dry	18	0.6
Pineapple			Made with Sparklet carbon dioxide		
Canned in sirup	1	120	capsule and distilled water	0	0
Frozen in sirup	1	38	White Rock	1	0.6
Juice, unsweetened, canned	0.5	140	Coca-Cola	1	52
Raw	0.3	210	Ginger ale	8	0.6
Plums			Lemon-lime soda	7	33
Canned in sirup	18	110	Orange Crush	2	100
Raw	0.6	170	Pepsi-Cola	15	3
Popcorn			Royal Crown Cola	5	2
Popped			Root beer	8	0.5
Oiled	3	240	Soup		
Oiled and salted	2000	240	Beef, canned, diluted as served	410	100
Pork			Tomato, canned, diluted as served	380	110
Lean, raw	58	260	Vegetable, canned, diluted as served	380	120
Salt	1800	27	Soybeans		
Postum			Dry	4	1900
Cereal beverage, dry	36	1300	Flour, solvent-extracted	1	1700
Instant, dry	71	2200	Spaghetti—See macaroni		
Potatoes			Spinach		
Chips	340	880	Canned	320	260
Sweet			Frozen	60	380
Canned	48	200	Raw	82	780
Raw, less skin	4	530	Squash, raw		
White			Acorn, less rind and seeds	0.4	260
Canned	350	240	Hubbard, less rind and seeds	0.3	240
Raw, less skin	0.8	410	White summer, less rind, with seeds	0.2	150
Poultry seasoning	26	840	Yellow summer, less rind, with seeds	0.6	200
Pretzel	1700	130	Squash, cooked, frozen	6	120
Prunes			Starch, corn	4	4
Canned in sirup	3	220	Strawberries		
Dried	6	600	Frozen, sweetened	2	180
Juice, unsweetened, bottled	2	260	Raw	0.8	180
Raw, with skin	0.7	210	Sugar		
Pumpkin			Light Brown	24	230
Canned	2	240	White	0.3	0.5
Raw, less rind and seeds	0.6	480	Sweetbreads—See pancreas and thymus		
Quail, raw			Tangerines		
Breast meat	35	160	Pulp and juice	2	110
Leg meat	44	190	Tapioca, drv	5	19
Quince, less skin and core, raw	0.7	290	Tea, India-Ceylon-Java blend, dry	4	1800
Rabbit, domesticated, raw			Thymus, beef, raw	96	360
Foreleg	47	370	Tomatoes		
Loin	34	400	Canned	18	130
Radish, with skin	9	260	Catchup	1300	800
Raisin			Juice, canned	230	230
Seedless	21	720	Raw, with skin	3	230
Raspberries			Tongue, beef, raw	100	260
Black	0.3	190	Tripe, pickled	46	19
Red	0.5	130	Tuna, canned	800	240
Rhubarb			Turkey, raw		
Frozen in sirup	2	160	Breast meat	40	320
Raw	1	70	Leg meat	92	310
Rice, dry			Turnips, raw		
Brown	9	150	Leaves	10	440
Flakes	720	180	White, less skin and tops	37	230
Polished and coated	2	130	Yellow (rutabaga), less skin and tops	5	260
Puffed	0.9	100	Veal, lean, raw	48	330
Wild (Zizania)	7	220	Vinegar		
Rum	2	3	Cider	1	100
Salmon			Distilled	0.6	15
Canned	540	300	Walnuts, raw		
Raw	48	410	Black	3	460
Sardines			English	2	450
Herring, canned in oil	510	560	Watermelon, pink part of fruit	0.3	110
Pilchard			Wheat		
Canned in natural sauce	760	260	Beeswing (outermost coats)	4	360
Canned in tomato sauce	400	320	Bran, crude	15	980
Sauerkraut, canned	630	140	Germ, crude	2	780
Sausage			Gluten	2	24
Bologna	1300	230	Winter, scoured—4 samples	2	370
Frankfurt	1100	220	Whiskey		
Pork	740	140	Blended	0.3	1
Scallop, frozen	150	420	Bonded	0.1	0.6
Shortening, vegetable			Wine		
Crisco	4	0	Port	4	75
Spry	0.4	0.2	Sauterne	10	87
Shrimp, raw	140	220	Worcestershire sauce	2100	480
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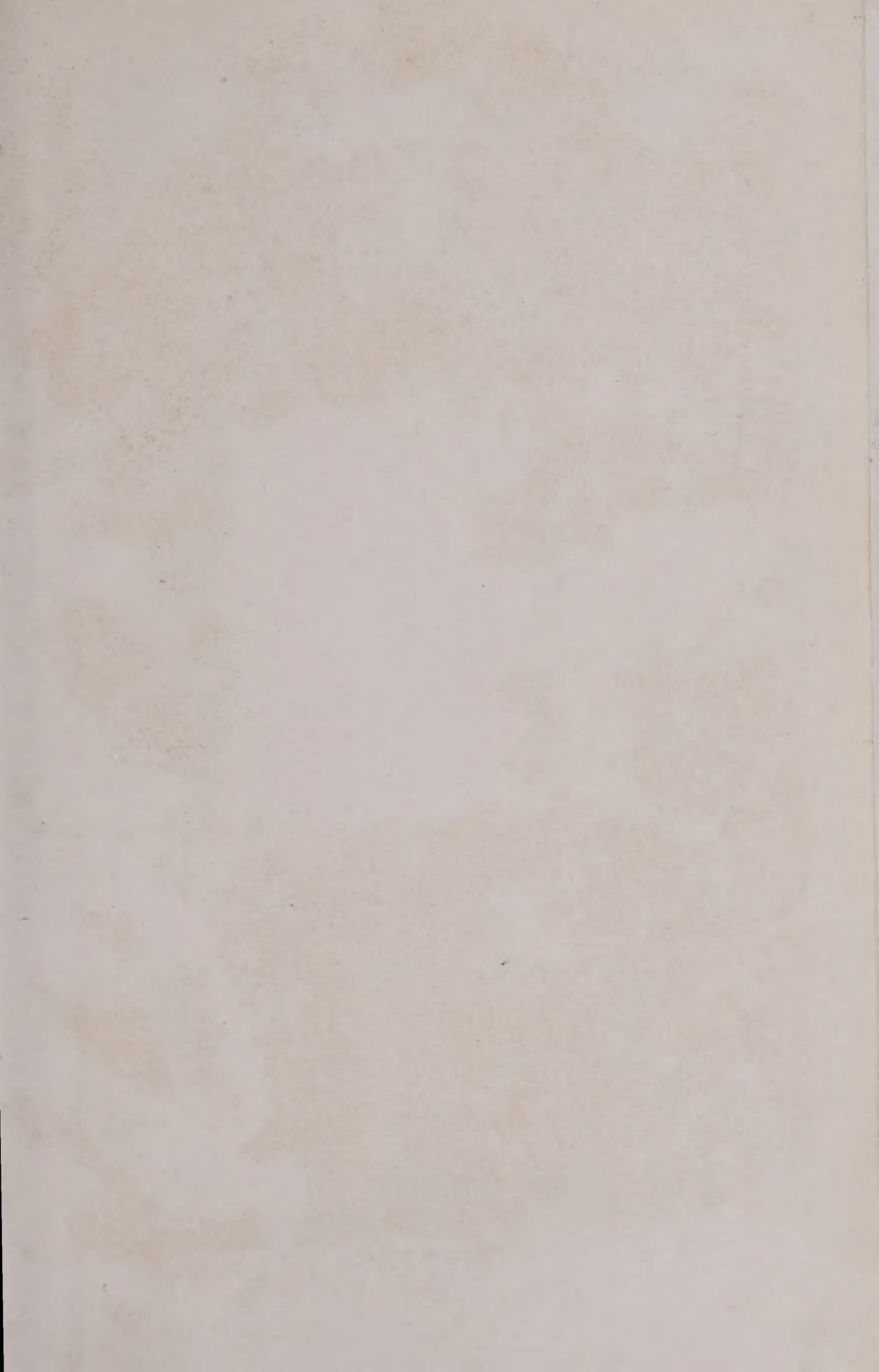
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